COVER PAGE

Official Study Title: Autologous Transplantation of Bone Marrow CD34+ Stem/Progenitor Cells After Addition of a Normal Human ADA Complementary DNA (cDNA) by the EFS-ADA Lentiviral Vector for Severe Combined Immunodeficiency Due to Adenosine Deaminase Deficiency (ADA-SCID)

EFS-ADA Protocol v12.0. dated 28-Mar-2018

NCT01852071

Autologous Transplantation of Bone Marrow CD34+ Stem/Progenitor Cells after Addition of a Normal Human ADA cDNA by the EFS-ADA Lentiviral Vector for Adenosine Deaminase (ADA)-Deficient Severe Combined Immunodeficiency (SCID)

IND Sponsor: Orchard Therapeutics, Ltd.

BB IND # 15440

Other Identifying Numbers: NIH OBA RAC #0910-1006

UCLA IRB # 11-002303

[redacted]

Version Number: 12.0 –post NIH RAC, FDA IND, and NHLBI DSMB reviews
Date: 28 March 2018

Statement of Compliance:

(i) Name of IND Drug: Autologous bone marrow CD34+ cells transduced with the lentiviral vector, EFS-ADA (OTL-101).

(ii) This is a Phase I/II Study.

(iii) This clinical investigation will not begin until an IND is in effect.

(iv) The Institutional Review Board (IRB) of the Office for Human Research Protection Program (OHRPP) at the University of California, Los Angeles (UCLA) will be responsible for the initial and continuing review and approval of this study. Any proposed changes will be reported to the IRB.

(v) The investigation will be conducted in accordance with all other applicable regulatory requirements.

(vi) The CLINICAL TRIAL Company, Ltd. (TCTC) will be responsible for monitoring the conduct and progress of the clinical investigation.

(vii) Orchard Therapeutics, Ltd. will be responsible under 312.32 for review and evaluation of information relevant to the safety of the drug.

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

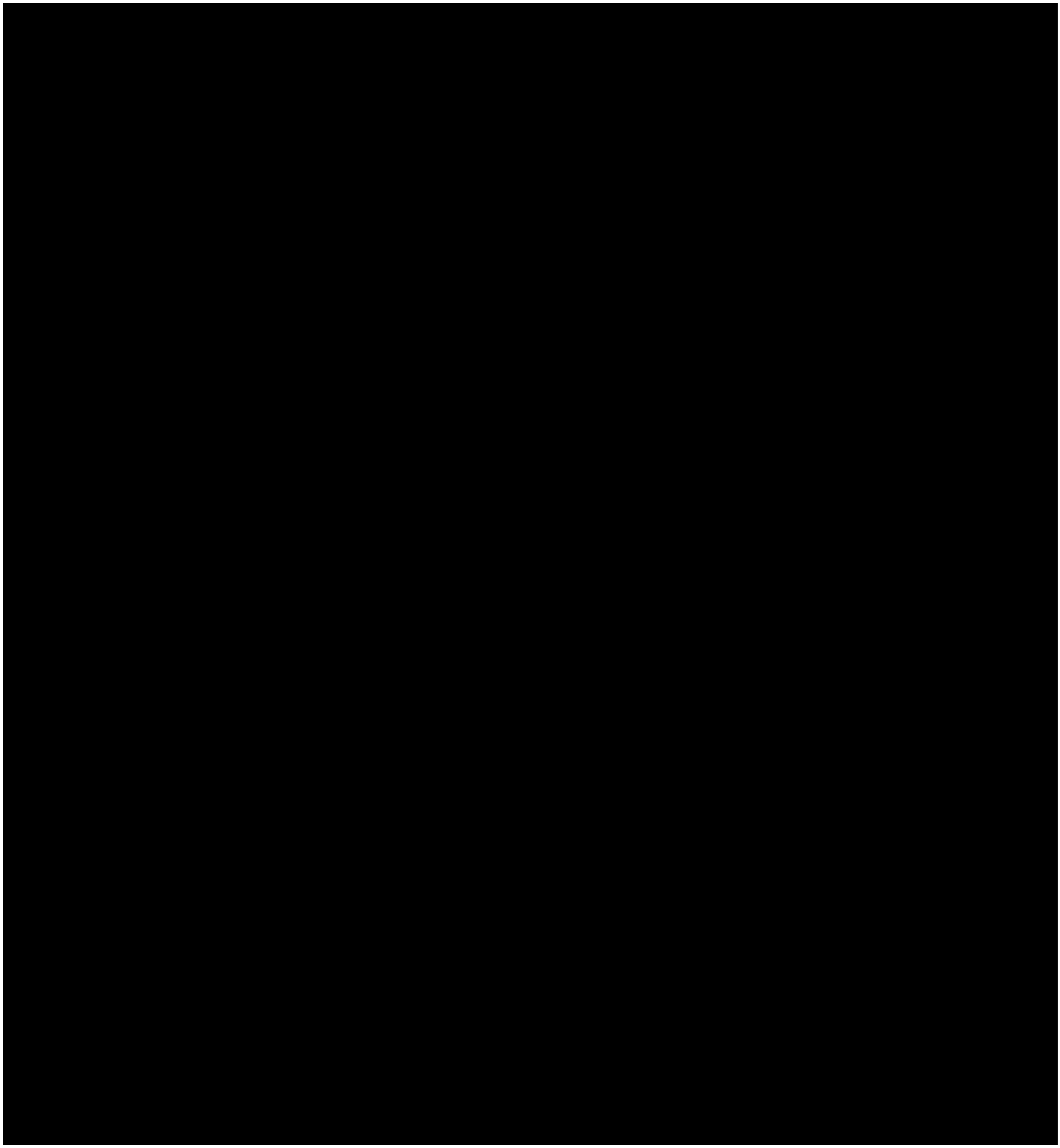


Table of Contents

List of Abbreviations

This list should be modified to include protocol-specific terms.

ADA	Adenosine Deaminase
ADA-SCID	ADA Deficient SCID
AE	Adverse Event/Adverse Experience
ANC	Absolute Neutrophil Count
BMT	Bone Marrow Transplant
CBC	Complete Blood Count
cDNA	Complementary Deoxyribonucleic Acid
CD	Cluster of Differentiation
CI	Clinical Investigator
CID	Combined Immunodeficiency
CRF	Case Report Form
dAXP	Deoxyadenosine
DCC	Data Coordinating Center
DSMB	Data and Safety Monitoring Board
EFS	Elongation Factor 1 alpha promoter (short)
EvFS	Event free survival
ERT	Enzyme Replacement Therapy
eLTCIC	Extended Long-Term Culture Initiating Cells
FDA	Food and Drug Administration
FWA	Federal-Wide Assurance
GALV	Gibbon Ape Leukemia Virus
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GOSH	Great Ormond Street Hospital
GVHD	Graft Versus Host Disease
HCG	Human Chorionic Gonadotropin
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HSC	Hematopoietic Stem Cell
HSCT	Hematopoietic Stem Cell Transplant
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICP	Intracranial Pressure
IDE	Investigational Device Exemption
IEC	Independent or Institutional Ethics Committee
Ig	Immunoglobulin
IL	Interleukin

List of Abbreviations

IND	Investigational New Drug
IO	Insertional Oncogenesis
IRB	Institutional Review Board
ISM	Independent Safety Monitor
IV	Intravenously
LAM-PCR	Linear Amplification-Mediated PCR
LPD	Lymphoproliferative Disorder
LTR	Long Terminal Repeat
MLV	Moloney Murine Leukemia Virus
MOI	Multiplicity of Infection
MFD	Matched Family Donor
MSD	Matched Sibling Donor
MUD	Matched Unrelated Donor
N	Number (typically refers to participants)
NCI	National Cancer Institute, NIH
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	National Institutes of Health
NK	Natural Killer Cells
NMDP	National Marrow Donor Program
NOD	Non-Obese Diabetic
OHRP	Office for Human Research Protections
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PEG-ADA	Pegylated Adenosine Deaminase
PGK	Phosphoglycerate Kinase
PHA	Phytohemagglutinin Stimulation Test
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
Plt	Platelet count
PO	Per os, by mouth
PRBC	Packed Red Blood Cells
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
QA	Quality Assurance
QC	Quality Control
RBC	Red Blood Cells
RCL	Replication Competent Lentivirus
RCR	Replication Competent Retrovirus
SAE	Serious Adverse Event/Serious Adverse Experience

List of Abbreviations

SCID	Severe Combined Immune Deficiency
SCF	Stem Cell Factor
SOP	Standard Operating Procedure
TNC	Total Nucleated Cells
UCBC	Umbilical Cord Blood Cells
UCBT	Umbilical Cord Blood Transplant
UCL	University College London
VSV	Vesicular Stomatitis Virus
WB	Western Blot
WBC	White Blood Cells
WNL	Within normal limits

1 OVERVIEW

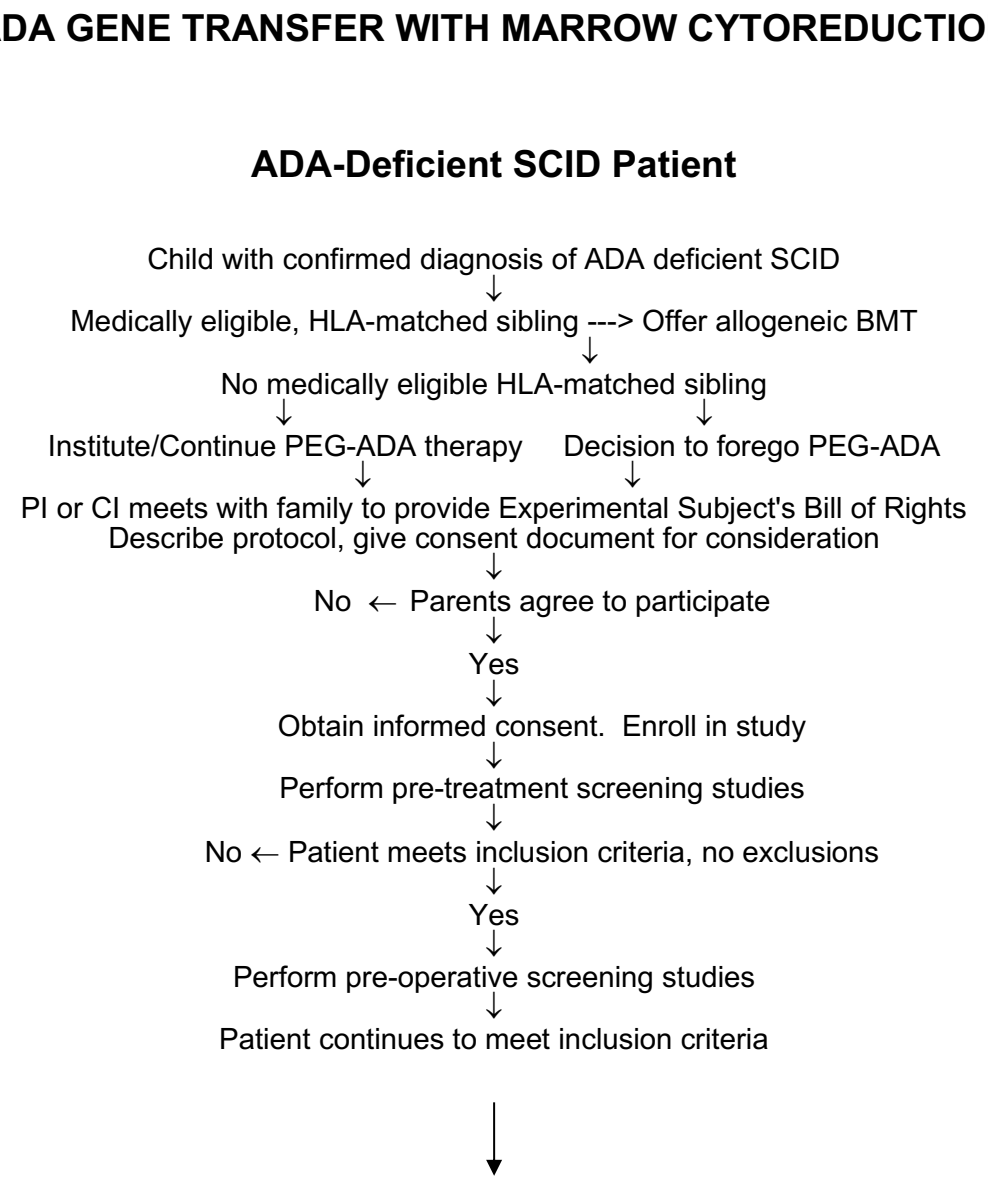
1.1 Protocol Summary

Full Title	Autologous Transplantation of Bone Marrow CD34+ Stem/Progenitor Cells after Addition of a Normal Human ADA cDNA by the EFS-ADA Lentiviral Vector for Adenosine Deaminase (ADA)-Deficient Severe Combined Immunodeficiency (SCID)
Short Title	Lentiviral Vector ADA Gene Transfer for ADA-Deficient SCID
Clinical Trial Phase	I/II
IND Sponsor	Orchard Therapeutics, Ltd.
Conducted By	University of California, Los Angeles and the National Institutes of Health
[redacted]	[redacted]
Sample Size	n=20
Study Population	Children older than 1.0 months who have ADA-deficient SCID
Accrual Period	5 years
Study Design	This is a controlled, prospective, non-randomized Phase I/II clinical trial to assess the safety (as primary end-point) and efficacy (as secondary endpoints) of autologous transplantation of CD34+ cells from the bone marrow (BM) of ADA-deficient SCID infants and children following human ADA cDNA transfer by the EFS-ADA lentiviral vector. Following provision of informed consent, enrolled subjects will be screened to determine full eligibility for participation over 1-2 weeks. Eligible subjects will be admitted to undergo harvesting of autologous BM under general anesthesia, with placement of a central venous access line, if not already present. If sufficient BM cells were collected to cryopreserve a “back-up sample” of at least $5x10^7$ total nucleated cells/kg (or $3x10^7$ mononuclear cells/kg) and also yield $\geq 1x10^6$ CD34+ cells/kg, the CD34+ cells will be cultured for EFS-ADA lentiviral vector-mediated transduction. The subjects will receive a single dose of busulfan (4 mg/kg) IV. At the end of transduction, the final cell product (autologous BM CD34+ cells transduced with the EFS-ADA lentiviral vector, OTL-101) will be assessed to determine if it meets release criteria, and if so, it will be infused IV. For subjects who have successfully received the final cell product, PEG-ADA ERT will be stopped at day +30 after transplant. The initial portion of the study will require the subject to be treated in the hospital for a typical duration of 10 days-6 weeks. After discharge from the hospital, the subject will be seen for interval history and examination by either their home physician, the PI or a CI and have blood

	drawn at months 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 21, and 24. Any medically-indicated interventions will be made at those visits. Subjects will then be consented for a long-term follow-up study with semi-annual to annual visits and lab draws over the subsequent 13 years.
Study Duration	Each patient will be followed for 24 months. There will be a separate study for long-term follow-up for these patients for years 3-13. The total follow-up period for these subjects will be up to 15 years.
Study Agent/Intervention Description	The Investigational New Drug to be tested is autologous BM CD34+ cells transduced *ex vivo* with a self-inactivating (SIN) HIV-1 based lentiviral vector: EFS-ADA (also referred to as OTL-101). The vector has been produced as cell-free lentiviral supernatant from the 293T cell line, and has an envelope pseudotype from the vesicular stomatitis virus (VSV). The vector will be used for *ex vivo* transduction of CD34+ progenitor cells from the BM of infants or children with adenosine deaminase (ADA)-deficient severe combined immunodeficiency (SCID). OTL-101 will be subsequently infused intravenously after a single non-myeloablative dosage of busulfan (4 mg/kg intravenously x1).
Primary Objective	The primary objectives are: • to examine the safety of autologous transplantation of bone marrow CD34+ cells transduced with the EFS-ADA lentiviral vector (OTL-101). The safety review will include the identification of grade III/IV serious adverse events (SAE). Subjects will also be monitored for clinical toxicities, replication competent lentivirus, and monoclonal expansion at regular intervals by a clinician, as well as standard blood counts and chemistries. • to estimate overall and event-free survival by 12 months where failure is defined by one of the following endpoints: death; reinstitution of PEG-ADA; or performance of an allogeneic BMT.
Secondary Objective	The secondary objectives will assess the efficacy of OTL-101, engraftment of transduced cells and the extent of ADA expression and immune reconstitution. The secondary outcomes will: 1. Assess overall and event free survival at 24 months, 2. Compare overall survival and event free survival at 24 months between patients treated with OTL-101 and patients treated with allogeneic HSCT, 3. Assess the extent of gene transfer in peripheral blood cells, 4. Assess ADA gene expression by measuring ADA enzymatic activity and adenine nucleotides, 5. Examine the effects of reconstituting ADA gene expression on immune function through serial examination of peripheral blood leukocytes and myeloid cells, 6. Assess immune reconstitution and infection rates, and 7. Assess use of immunoglobulin replacement therapy.

Endpoints	**<u>Primary Safety Endpoint</u>:** Incidence and grade of Serious Adverse Events (SAE). Serious adverse events may be manifest as: • clinical toxicities • exposure to replication-competent lentivirus (RCL) • development of monoclonal expansion or leukoproliferative complications from vector insertional effects. **Primary Efficacy Endpoint:** Overall/event-free survival (survival without need for BMT or PEG-ADA). Overall/event-free survival will be determined for each subject by 12 months where failure is defined by one of the following endpoints: death; reinstitution of PEG-ADA; or performance of an allogeneic BMT. **Secondary Endpoints include:** • Overall survival and event free survival at 24 month • Efficacy of gene transfer/engraftment of HSC • ADA expression, enzyme activity and detoxification • Effects on ADA reconstitution on immune phenotype and function (the need for IgRT will be assessed under this category) • Immune reconstitution • Infection rates

1.2 Schematic of Study Design:



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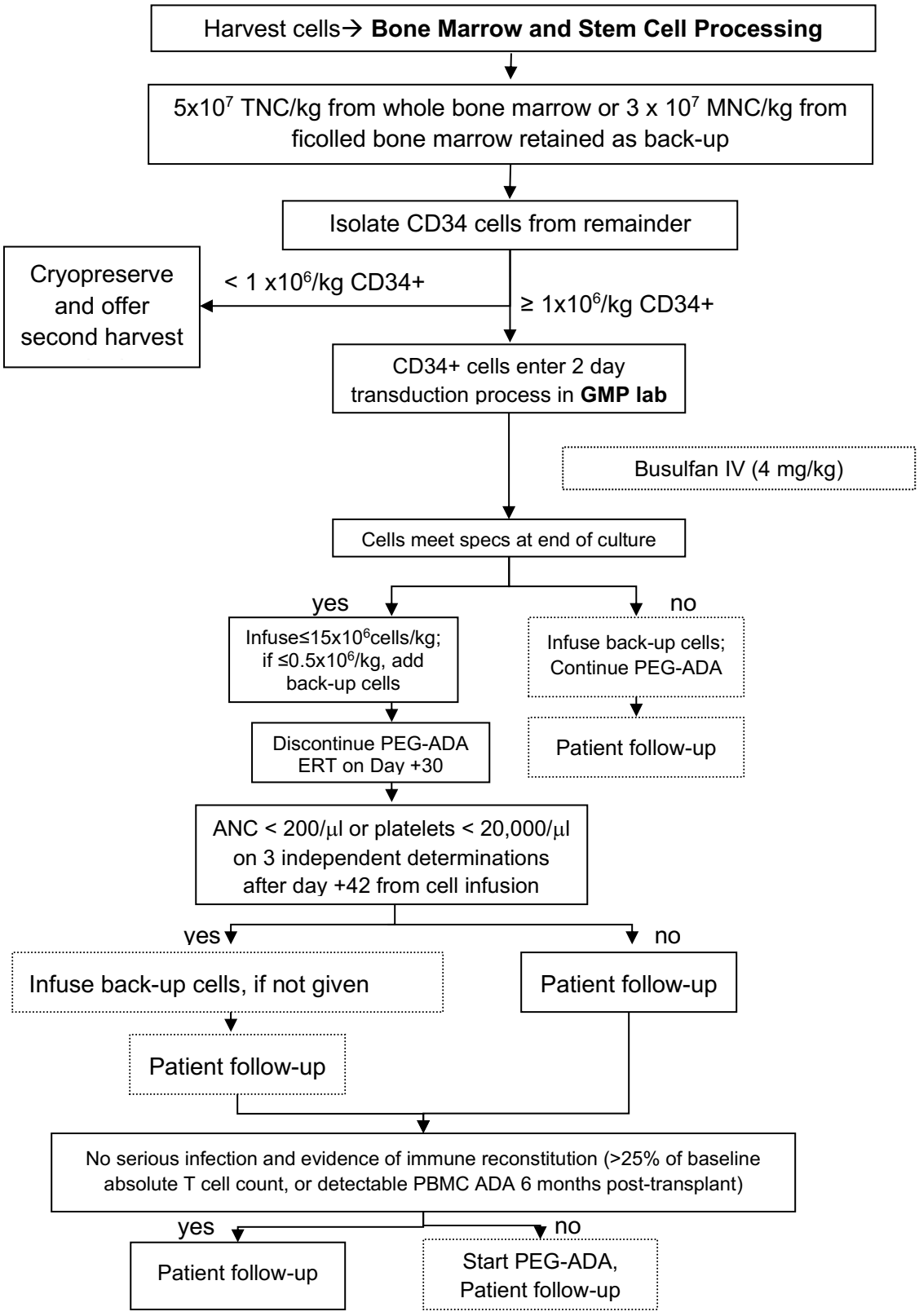
Harvest cells→ **Bone Marrow and Stem Cell Processing**
5x10^7 TNC/kg from whole bone marrow or 3 x 10^7 MNC/kg from ficolled bone marrow retained as back-up
Isolate CD34 cells from remainder
< 1 x10^6/kg CD34+
Cryopreserve and offer second harvest
≥ 1x10^6/kg CD34+
CD34+ cells enter 2 day transduction process in **GMP lab**
Busulfan IV (4 mg/kg)
Cells meet specs at end of culture
yes
no
Infuse≤15x10^6cells/kg; if ≤0.5x10^6/kg, add back-up cells
Infuse back-up cells; Continue PEG-ADA
Discontinue PEG-ADA ERT on Day +30
Patient follow-up
ANC < 200/μl or platelets < 20,000/μl on 3 independent determinations after day +42 from cell infusion
yes
no
Infuse back-up cells, if not given
Patient follow-up
Patient follow-up
No serious infection and evidence of immune reconstitution (>25% of baseline absolute T cell count, or detectable PBMC ADA 6 months post-transplant)
yes
no
Patient follow-up
Start PEG-ADA, Patient follow-up

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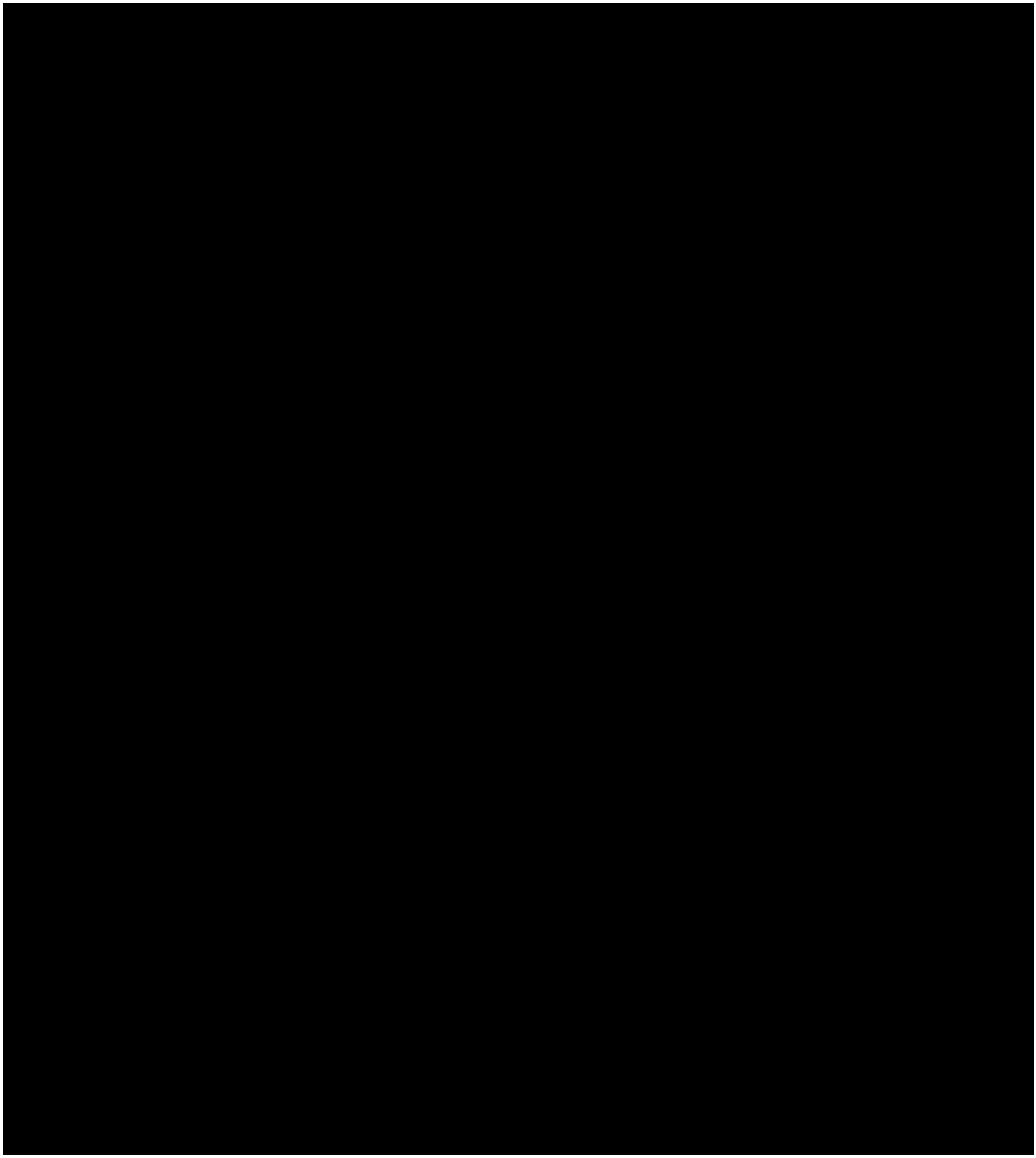
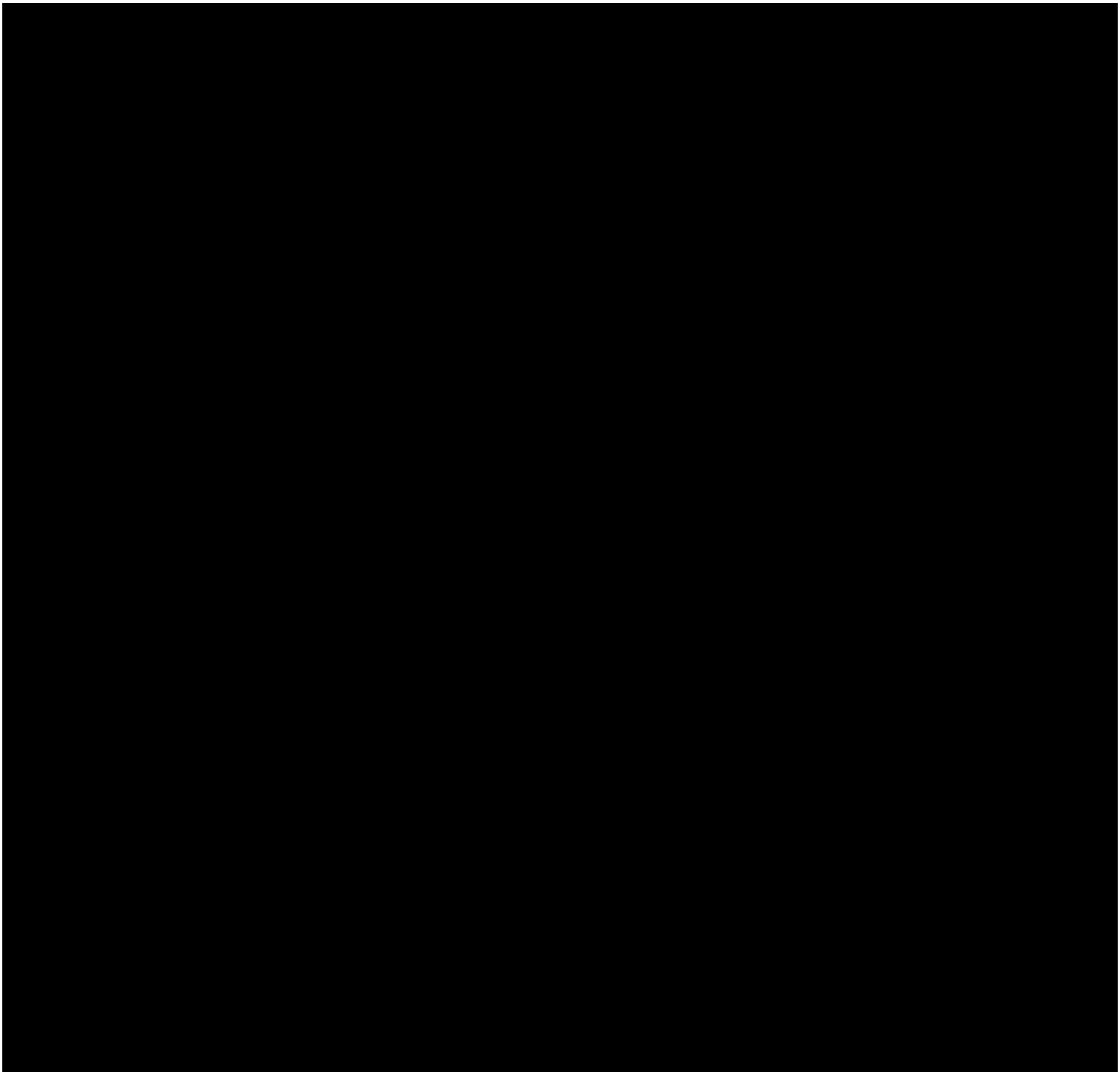




2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

2.1.1 Severe combined immunodeficiency (SCID)

ADA acts on both adenosine and deoxyadenosine, producing inosine and deoxyinosine (Hershfield and Mitchell, 1995; Hirschhorn, 1993). In the absence of ADA, deoxyadenosine can be phosphorylated, particularly by lymphoid cells, which have high levels of the enzyme deoxycytidine kinase. The resulting dATP pool expansion has been shown to inhibit DNA replication and repair, and to induce apoptosis in immature thymocytes. In addition, deoxyadenosine inactivates the enzyme S-adenosylhomo-cysteine (SAH) hydrolase; SAH accumulation inhibits transmethylation reactions, which may contribute to immunodeficiency. It has been speculated that effects of extracellular accumulated adenosine acting through its various receptors may also play a role in pathogenesis.

A minority (10-15%) of ADA-deficient patients does not present with the classical phenotype of “severe combined immunodeficiency” (SCID), but with a milder and variable degree of immune dysfunction that delays their diagnosis beyond the first year of life (delayed onset) or the first decade (late onset). Although abnormal, immunity is partially conserved in these patients who are commonly classified as being affected with “combined immunodeficiency” (CID) until a definitive diagnosis is made. The clinical presentation of these patients is characterized mostly by recurrent lung infections that occur at higher frequency than in normal subjects, but that usually respond to therapy, which can mask the true underlying cause for many years. The immunodeficiency of these patients may be adequately controlled with antibiotics and immune globulin prophylaxis, or they may be treated in the same manner as early onset ADA-Deficient SCID.

2.1.2 Adenosine deaminase (ADA)-deficient SCID

Severe combined immunodeficiency (SCID) has been recognized for over four decades as an inherited disorder in which patients have profoundly defective immunity with essentially no T lymphocyte function and minimal to absent B cell function (Kohn and Weinberg, 1996; Buckley, 2004). SCID patients typically present for medical attention between 3-9 months of age with multiple, recurrent severe infections with common childhood pathogens (encapsulated bacteria, respiratory syncytial virus, parainfluenza virus) or opportunistic organisms (*Candida albicans*, *Pneumocystis jirovecii*, cryptococcus). Related findings often include failure to thrive and wasting, persistent diarrhea, and delayed growth and dentition.

The clinical syndrome of SCID is due to a variety of specific genetic lesions, each of which impairs normal T cell development and function. The first specific genetic cause of SCID to be identified was deficiency of the enzyme adenosine deaminase (ADA) (Giblett et al, 1972). It is estimated that ADA deficiency accounts for 15-20% of cases of SCID. Other known genetic lesions resulting in SCID include: mutations in the IL-2 receptor gamma chain (γ_c), (responsible

for X-linked SCID); defects in Jak3 kinase and other components of T cell intracellular signaling pathways; the inability to produce interleukin-2 (IL-2); defects in components of the T cell antigen receptor complex; defects in the recombinase system which is responsible for rearrangement of immunoglobulin and T cell receptor genes; and impaired expression of HLA antigens (bare lymphocyte syndrome). Once a definitive diagnosis of ADA deficiency is made, patients may be treated with a hematopoietic stem cell transplant (HSCT), which includes HLA-identical matched sibling donor bone marrow transplant (BMT) or haplo-identical parental T cell-depleted BMT, or in some cases a matched unrelated adult donor BMT or matched unrelated cord blood HSCT. However, if a matched sibling donor is unavailable, most ADA-deficient patients are treated with ADA enzyme replacement therapy (ERT). These treatment options and outcomes are discussed in detail below.

2.1.3 Treatment Options for ADA-Deficient SCID

2.1.3.1 Allogeneic Bone Marrow Transplantation (BMT)

a. Matched sibling donor BMT

Bone marrow transplantation was first successfully applied in the treatment of a patient with SCID in 1968 (Gatti, et al, 1968). ADA deficiency was recognized as a cause of SCID in 1972 and patients with ADA-deficient SCID were successfully transplanted at that time (Giblett, et al, 1972; Parkman, et al, 1975). For SCID patients with an HLA-matched sibling donor, BMT is the treatment of choice. Barring patients with severe pre-existing infections, greater than 90% of SCID patients (with or without ADA deficiency) can be cured with BMT from a matched sibling. Due to the lack of immune reactivity, a matched sibling BMT for SCID is accomplished quite easily; a modest amount of donor bone marrow (e.g. 5 x 10^7 cells/ kg) is taken directly from the donor and given to the SCID recipient without the use of cytoablation or immune suppression. The majority of patients treated by matched BMT will develop a normal immune system comprised of donor-derived T lymphocytes.

b. Haplo-identical, parental T cell depleted BMT

However, only ~20% of SCID patients will have a matched sibling donor. An alternative treatment which has been developed is the use of haplo-identical (parental) bone marrow, depleted of mature T lymphocytes. The results with T cell-depleted BMT for SCID patients have gradually improved, so that 60-80% of patients will survive with gain of improved-to-normal immune function (Fischer, et al, 1990; O'Reilly, et al, 1989; Buckley, et al, 1999; Filipovich, 2008; Cowan, 2008, Griffith, 2009). However, despite T cell depletion of the donor marrow, patients can suffer graft versus host disease (GVHD), requiring further immunosuppressive therapy. Some patients will recover only moderate immune function, with B cell deficiency and hypogammaglobulinemia seen in up to half of the patients. Additionally, immune recovery is a slow process, taking 4-12 months, during which time the patients are vulnerable to opportunistic infections.

Buckley and co-workers have reported their results using haplo-identical T-depleted BMT to treat 77 SCID patients, of which 60 of 77 survived (78%). Of the total transplants: 74 of 77 of

these received the transplants without the use of cytoablative therapy or GVHD prophylaxis; 3 received unrelated donor cord blood transplants (2 with pre-conditioning and post-GVHD prophylaxis) after unsuccessful haplo transplants; 19 required at least a second cell infusion (with a few subjects getting three or four transplants); 21 of 60 were stated to have "some donor B cells,"; and 45 of the total 72 survivors continue to receive IVIg.

Specifically, 13 subjects had ADA-deficiency as the genetic basis of their SCID and 11 of these survived. Of the 13 patients, 9 received haplo-identical T cell-depleted transplants and 4 received matched sibling transplants. Of the 9 receiving haplo-identical T cell-depleted transplants, 7 were alive. Of the 7 surviving patients, 6 have donor engraftment (66%) and one subject is receiving PEG-ADA after rejection of two paternal haplo-identical T-depleted transplants. Of the 11 surviving ADA-deficient subjects, only 3 had evidence of donor B cell production and 5 were still receiving IVIg. From the publication, it is not possible to determine how many of the ADA-deficient subjects were infants, defined as less than 3 months of age at the time of transplant.

c. Unrelated donor BMT

Using unrelated or haplo-identical (parent) bone marrow from adult donors, the results for ADA-deficient SCID are consistently significantly worse than for other genetic forms of SCID. For example, in a large retrospective review of the pan-European experience, survival for other types of SCID patients was 77% with HLA-identical donors compared to 54% for HLA-mismatched donors. However for ADA-deficient SCID patients, survival was 88% with HLA-identical donors compared to only 29% with HLA-mismatched donors (Antoine, et al, 2003).

A consensus conference held in 2006 accumulated the following outcome data on allogeneic HSCT for adenosine deaminase-deficient SCID from 4 major centers in Europe and 1 US center (Booth, et al, 2007).

Table 1. HSCT transplant outcome for ADA-SCID

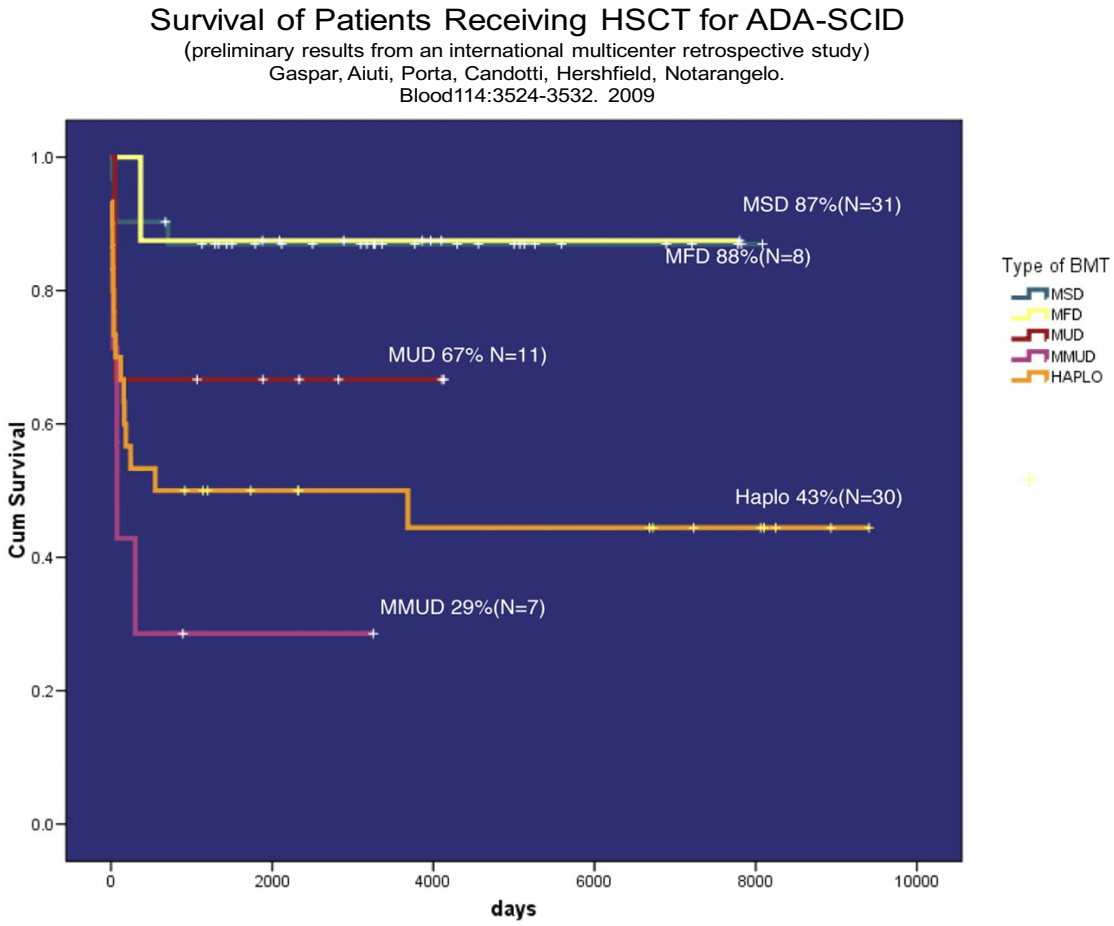
	Total	MSD/MFD		MUD		Haploidentical		
		Number	Alive[a]	Number	Alive[a]	Number	Alive	Alive + T engraftment
London	31	13	11	5	3	13	3	3
Ulm	15[b]	7	7	2	1	6	4	4
Brescia	4	3	3	1	1	0	0	
Paris	7	3	2	0	0	4	0	
Duke[c]	23	4	4	0	0	19	14	7
Total	80	30	27 (90%)	8	5 (63%)	42	21 (50%)	14 (33%)

MSD—matched sibling donor.
MFD—matched family donor.
MUD—matched unrelated donor.
[a] All patients alive with T-cell engraftment.

[b] Two patients from Munich.
[c] All patients transplanted without conditioning.

More recently, these results were supplemented by ascertainment of the outcomes in additional cases of allogeneic BMT for ADA-deficient SCID (Gaspar, et al, 2009) (see Figure 1). These results demonstrate that allogeneic BMT other than with a matched related sibling donor produce sub-optimal rates of survival and immune reconstitution.

Figure 1. Survival of patients receiving HSCT for ADA-SCID



d. Umbilical cord blood HSCT

There are few published series reporting the results of unrelated umbilical cord blood hematopoietic stem cell transplant (**UCBT**) for SCID patients of any genetic types and only four for ADA-SCID. Knutsen et al. (2010) performed 3 UCBT for non-ADA SCID patients. Other than grade I GVHD, they are all doing well. In another study, of 9 SCID patients that were treated with UCBT, 4 had ADA-deficient SCID. 2/4 of the ADA-deficient patients died and one of the surviving patients had grade III GVHD. All of the other 5 patients, who had other forms of SCID, survived (Bhattacharya, et al, 2005). This series was listed in a recent review and a fifth ADA-SCID patient had since been transplanted with umbilical cord blood as the donor source. This brought the

overall survival to 40% (Gennery and Gant, 2007). Another series of 8 non-ADA-deficient SCID had 2/8 survive, but 7/8 experienced GVHD and 2 were grade 3-4.

Altogether, these reports show that following unrelated umbilical cord blood transplants, 14/16 (87.5%) SCIDs (non-ADA) are alive, but only 2/5 (40%) ADA-deficient SCIDs are alive. These are small numbers of patients, but the results are consistent with the larger numbers of SCID recipients of MUD adult products, showing worse outcome for the ADA-deficient form of SCID when compared to the other forms SCID. Furthermore, the recipients of unrelated cord blood receive high dose chemotherapy conditioning (e.g. busulfan 16 mg/kg plus cyclophosphamide 200 mg/kg and ATG) and require post-transplant GVHD prophylaxis (Cyclosporine A and methylprednisolone, despite which, cord blood HSC recipients often have frequent low grade GVHD and occasional high grade GVHD.

e. Recent multinational report on outcomes from allogeneic HSCT for ADA- deficient SCID:

[Hassan et al. Blood e-pub July 2012]. (Paraphrased from the Abstract):

In this multicenter retrospective study, the authors analyzed outcome of HCT in 106 patients with ADA-SCID who received a total of 119 transplants. HCT from matched sibling and family donors (MSD, MFD) had significantly better overall survival (OS) (86% and 81%) in comparison to HCT from matched unrelated (66%; $p<0.05$) and haploidentical donors (43%; $p<0.0001$). Superior OS was also seen in patients who received unconditioned transplants in comparison to myeloablative procedures (81% vs. 54%; $p<0.003$) although in unconditioned haploidentical donor HCT, non-engraftment was a major problem. Long term immune recovery showed that regardless of transplant type, overall T cell numbers were similar although a faster rate of T cell recovery was observed following MSD/MFD HCT. Humoral immunity and donor B cell engraftment was achieved in nearly all evaluable surviving patients and was seen even after unconditioned HCT. These data detail for the first time the outcomes of HCT for ADA-SCID and demonstrate that if patients survive HCT, long term cellular and humoral immune recovery is achieved. These outcomes confirm many previous smaller reports on the rates of success and complications for different types of allogeneic HSCT for ADA-deficient SCID.

Thus, while T cell-depleted or unrelated adult donor or cord blood HSCT have become useful treatment modalities for ADA-deficient SCID patients, they are still beset by a number of serious limitations. Because of these limitations, haploidentical or matched unrelated BMT are generally not offered as options for ADA-SCID. The two conventional treatment options are HLA-identical related BMT for those with available matched siblings and ADA enzyme replacement therapy in the form of PEG-ADA for most others.

2.1.3.2 ADA Enzyme Replacement Therapy

In the mid-1970's, it was observed that transfusion of packed red blood cells from normal donors into ADA-deficient patients could cause a partial improvement in immune function, including a rise in the lymphocyte count (Polmar, et al, 1976). It was postulated that ADA within the donor RBC was acting to detoxify adenosine and deoxyadenosine metabolites, thereby protecting developing T lymphocytes. However, the responses to the RBC transfusions were only seen in a minority of patients and were typically modest in magnitude and duration.

Based upon the experience with RBC transfusions, a far more effective means of ADA enzyme replacement was developed. Bovine ADA may be isolated in relatively large quantities, but has a half-life of only minutes when injected parenterally and may be immunogenic upon repeated exposures. It had previously been shown that conjugation of proteins to polyethylene glycol (PEG) leads to greatly prolonged serum survival time and decreased immunogenicity. A pharmaceutical preparation of bovine ADA conjugated with polyethylene glycol (PEG-ADA, ADAGEN™) was approved by the US FDA in 1990 for treating ADA deficiency. It has been used to treat over 130 patients, often those considered to be too ill to safely undergo haplo-identical bone marrow transplantation (Hershfield et al, 1992; updated in Gaspar, 2009). PEG-ADA avoids the risks of red cell transfusion, and the amount of ADA enzyme activity provided by one or two weekly intramuscular injections of PEG-ADA exceeds by as much as 10 fold the amount that can be supplied by repeated RBC transfusions. PEG-ADA has been well tolerated, with no reports of allergic reactions thus far.

Treatment with PEG-ADA consistently corrects metabolic abnormalities due to ADA deficiency. Lymphocyte counts and immune function begin to improve after 2 to 4 months of treatment. Most patients have done well clinically (overall mortality is <20%, and ~10% in patients treated more than 6 months) (Hershfield & Mitchell, 1995; Gaspar 2009). Recovery of immunologic function on PEG-ADA therapy has been variable, and about 20% of patients, primarily those with the most severely compromised immune function, have had minimal improvement in lymphocyte counts and function. We reported long-term (4-11 years) follow-up on a cohort of ADA-deficient SCID patients treated with ADA-GEN at Children's Hospital Los Angeles and observed that T lymphocyte counts never reached into the normal range and had progressively declined to values of 100-400 over the duration of treatment (Chan et al, 2005). A few patients on PEG-ADA developed autoimmune hemolytic anemia, which was eventually fatal. Autoimmune disorders are seen in some patients with incomplete immune function and may represent the presence of immune effector cells without an appropriate level of immune regulatory cells. Others have died from progressive pulmonary insufficiency and/or have developed lymphoproliferative disorders or hepatocellular carcinoma.

The other major limitation to PEG-ADA therapy is the high cost. Treatment of a child with weekly PEG-ADA injections may cost between $200,000-500,000 per year. Because PEG-ADA therapy is palliative and not curative, injections must be continued throughout the life of the patient. The high cost of this treatment may limit access and create severe financial burdens on patients and their families.

2.1.4 Gene Therapy for ADA-Deficient SCID

2.1.4.1 ADA Gene Therapy Using T Lymphocytes

Gene therapy as a new alternative treatment for ADA-deficient SCID has been under investigation for more than two decades. The first clinical trial of human gene therapy for ADA-deficient SCID targeted the corrective ADA gene to peripheral blood T cells. Pre-clinical studies performed at the NIH in the mid-1980's demonstrated that introducing a normal human ADA cDNA (using a retrovirus as the gene delivery vector) into cultured T lymphocytes from patients with

ADA-deficient SCID resulted in the production of normal levels of ADA enzyme (Kantoff et al, 1986). In cell culture, the "cured" cells were able to survive in the presence of levels of deoxyadenosine that were toxic to the parental ADA-deficient cells. Studies performed in Italy demonstrated that ADA-corrected human T lymphocytes survived significantly longer *in vivo* in immune deficient mice compared to ADA-deficient cells (Ferrari et al, 1991).

Based upon these experimental results, a trial of gene therapy for ADA deficiency was performed in two patients at the NIH, beginning in 1990 (Culver et al, 1991; Blaese et al, 1995; Mullen et al, 1996). Peripheral blood T lymphocytes were collected by leukopheresis, transduced in the laboratory with the ADA gene, expanded in numbers by stimulating with growth factors and then re-infused into the patients. The procedures of leukopheresis, transduction and reinfusion were repeated at 1-2 month intervals over the course of two years. Two children were treated by this method with no evidence of toxicity. The presence of cells containing the inserted ADA gene was documented in both patients, although one patient had a significantly greater number of transduced cells than the other. In at least one of the patients, ADA-transduced T lymphocytes remain at a relatively stable level of 30-50% of total PBL, six years after the last cell infusion; this observation shows that T lymphocytes subjected to *ex vivo* transduction are capable of extended *in vivo* survival (Muul et al, Blood 2003). Both patients had significant increases in numbers of circulating T lymphocytes and some evidence of improved immune function. However, they remain on PEG-ADA enzyme replacement, making it difficult to attribute immunologic functions to the gene transfer. An ADA-deficient SCID patient in Japan was treated under this same protocol with good ADA gene transfer achieved (Onodera et al, 1998; Misaki, 2001).

2.1.4.2 Gene Therapy for ADA-Deficient SCID using Hematopoietic Stem Cells

a. Gene Transfer into Hematopoietic stem cells

One drawback to gene therapy using T lymphocytes is that the immunologic repertoire of the transduced lymphocytes may be restricted to those specificities present in the population of cells which were treated; responses to newly encountered antigens may not be possible. Additionally, these cells may have a finite life-span (months to years), which may necessitate repeated ADA gene transfers to be performed to sustain a therapeutic level of transduced cells.

Insertion of the ADA gene into hematopoietic stem cells (HSC), rather than mature T lymphocytes, may produce a long-lived effect. HSC are cells which produce all blood cells by a complex process of proliferation and differentiation. HSC are long-lived and can function for the life of the individual after transplantation. For children and adults, HSC are found primarily in the bone marrow, but HSC are also present in the umbilical cord blood at birth. Genetic correction of a patient's HSC may provide a continuous, enduring source of gene corrected mature blood cells, including T lymphocytes.

From more than twenty years, γ-retroviral-mediated gene transfer into hematopoietic stem cells can routinely transduce the majority of stem cells in murine gene transfer/BMT models. However, work in large animal models (canine and rhesus) in the 1990's could only achieve 0.1-1.0% stem cell transduction (Kohn, 1995; Dunbar, 1996). The initial sets of clinical trials of gene transfer into autologous hematopoietic cells for ADA-deficient SCID and other disorders

performed to that time had also shown extremely limited abilities to transduce long-lived human stem cells, with marking of only 0.1-1% of cells seen. The reasons for the greater difficulty in performing gene transfer into stem cells from the large animals and human subjects were not fully understood; possible explanations include lower numbers of receptors for the amphotropic virus envelopes used, intracellular blocks to virus integration, or a lower percentage of stem cells in active cell cycle which can be transduced by γ-retroviral vectors.

Over that time period, incremental improvements in γ-retroviral-mediated gene transfer into human hematopoietic stem cells (HSC) were achieved, using GALV pseudotypes, "mobilized BM," recombinant fibronectin support, new cytokines (Flt-3 ligand, thrombopoietin), and manipulation of cell cycle kinetics (Kiem et al, 1997; Dunbar et al, 1996; Hannenberg et al, 1996; Shah et al, 1996; Dao et al, 1997; Dao et al, 1998; Piacibello et al, 1997). Combinations of these techniques resulted in modest, yet significant, increases in gene marking in primate stem cell transplant models (e.g. 10%, up from the previous ceiling of 0.1-1.0%) (Kiem et al, 1998; Tisdale et al, 1998). Based on these methodological advances, a second generation of clinical trials of gene therapy for both ADA-deficient SCID as well as XSCID and other disorders were begun in this decade.

b. Gene therapy for ADA deficiency using hematopoietic stem cells

At least 50 ADA-deficient SCID patients have now been treated by insertion of the ADA gene into their bone marrow cells (see Table 2, below).

Table 2. Clinical Trials of Gene Therapy for ADA-deficient SCID (as of June 2011)

Year	Site	Cell Type	Vector	PEG-ADA	Pre-transplant Conditioning	Clinical Benefit (#benefited/ #treated)
1990	Bethesda, U.S.	T	RV	Continued	n.a.	No (0/2)
1993	Milan, Italy	T & BM	RV	Continued	No	No (0/2)
1993	Netherlands/UK	BM	RV	Continued	No	No (0/3)
1993	L.A./Bethesda, U.S.	UCB	RV	Continued	No	No (0/3)
1995	Milan, Italy	T	RV	Continued	n.a.	No (0/3)
1996	Hokkaidō, Japan	T	RV	Continued	n.a.	No (0/1)
2000	Milan, Italy	BM	RV	No	Busulfan (4 mg/kg)	Yes (15/18)
2004	Hokkaidō, Japan (Otsu *et al*)	BM	RV	No	None	No (2/2)
2002	London, UK	BM	RV	No	Melphalan (140 mg/m2)	Yes (3/6)
2001	L.A./Bethesda, U.S.	BM	RV	Continued	None	No (0/4)
				No	Busulfan (75-90 mg/m2)	Yes (3/6)

2009	L.A./Bethesda, U.S.	BM	RV	No	Busulfan (4 mg/kg)	Yes (4/5)

T = peripheral blood T lymphocytes, BM = bone marrow, UCB= umbilical cord blood, RV = γ-retroviral vector, n.a. = not applicable

An initial set of clinical trials performed in Europe and the US in the 1990's in which γ-retroviral vectors were used to introduce the human ADA cDNA into bone marrow CD34+ cells from ADA-deficient patients were ineffective. All of the studies only were able to achieve very low numbers of ADA-gene corrected blood cells, generally at the limits of detection by PCR, and in most cases these cells were only present for the initial months after the procedure (Ferrari et al 1992; Bordignon et al, 1993; Hoogerbrugge et al, 1992; Kohn 1993). In all of these trials, patients continued to receive PEG-ADA enzyme therapy because of the ethical concerns about stopping an approved efficacious medication to test an unproven approach. While the PEG-ADA sustained the immunity of these patients, it may have blunted the putative selective survival advantage for ADA-expressing lymphocytes that may support the amplification of lymphoid cells produced by gene-transduced stem/progenitor cells. Additionally, the patients did not receive any pre-transplant conditioning with chemotherapy, which may have facilitated the engraftment of the transplanted gene-modified stem/progenitor cells, again due to the potential risks and unknown prospects of benefit.

A clinical trial of gene therapy for ADA deficiency carried out in Italy starting in ~2000, initially reported on two patients who did not have access to PEG-ADA and who were treated with gene-corrected autologous CD34+ bone marrow cells after receiving chemotherapy with Busulfan at the dose of 4 mg/kg (Aiuti, 2002). In this case, normalization of T cell numbers and function was observed as soon as 6 months after treatment in the first patient and partial improvement of T cell counts was observed in the second patient. Both patients are well with normal growth and development (Aiuti et al 2001; Aiuti et al 2002). An additional 10 subjects have been enrolled in Milan and six in London, and early reports demonstrate that most of them have reconstituted immunity (Aiuti A et al., 2009; Gaspar HB, et al, 2006). These results are unprecedented for gene therapy of ADA deficiency and represent a dramatic improvement over previous clinical trials. In the Italian and London trials, chemotherapy was administered to aid engraftment of gene-corrected cells. We have recapitulated these results in our trials, as described below and will be discussed in detail in section 2.1.2. No adverse reactions from the vectors have been noted to date in these ADA deficient patients.

2.1.5 Summary of Previous Clinical Studies with γ-Retroviral Vectors by Investigators

2.1.5.1 CHLA/NIH trial of gene transfer into umbilical cord blood CD34+ cells from ADA-deficient SCID infants (1993)

We have performed a clinical gene therapy study with direct relevance to the studies proposed here (Kohn et al, 1995). Three infants were diagnosed prenatally with adenosine deaminase (ADA)-deficiency, in families with previous affected infants with severe combined immunodeficiency (SCID). In 1993, these infants were infused with γ-retroviral vector-transduced

umbilical cord blood CD34+ cells on their fourth day of life. Each infant was immediately started on enzyme replacement therapy with PEG-ADA. As a result, they had normalization of the levels of deoxyadenosine metabolites, which were elevated at birth, and improvements of their T cell numbers and function over the first few months. One patient was kept on a fixed dose of PEG-ADA so that the effective dose/kg decreased as he grew; the other two patients were maintained on a constant dose/kg (adjusted as they grew) for the first 18 months (through Dec 94) and were then lowered to half this dose for the subsequent time.

In summary, this trial of gene transfer to ADA-deficient CD34+ cells showed that:

1. Long-lived hematopoietic progenitor (stem) cells among the CD34+ cells from umbilical cord blood can be transduced by γ-retroviral vectors, engraft without prior administration of cytoablative conditioning, and produce gene-containing peripheral blood leukocytes for at least 10 years.

2. There is selective accumulation of gene-containing T cells upon reduction of the PEG-ADA dosage.

3. Expression by the LASN vector in resting CD3+ T lymphocytes is poor (<1% of that seen in Jurkat cells). Expression increases significantly upon stimulation of the T cells with PHA (to approximately the same level as in Jurkat cells).

4. While there was no evidence that either B lymphocytes or NK cells containing the ADA gene selectively accumulate, both of these cell types were rapidly lost upon withdrawal of PEG-ADA.

5. In one subject, complete cessation of PEG-ADA for two months did not demonstrate that he had protective immune function solely from gene-corrected cells. Thus, the low level of transduced progenitors present in this patient was not sufficient to sustain immune function following PEG-ADA withdrawal during the observation period. Potentially, higher frequencies of transduced progenitor cells may allow long-lasting, protective immune function independently of exogenous enzyme replacement.

6. No abnormal lymphoproliferation was detected in these 3 umbilical cord blood enrollees.

7. Retrospective analysis by LAM-PCR showed that one of the subjects had a nearly-monoclonal pattern of marking, with a single predominate vector integrant present and stable over more than eight years, without cell expansion. This predominant integrant was seen in peripheral blood T cell clones with different patterns of T cell receptor rearrangement, indicating that transduction was of a pre-thymic stem or progenitor cell (Schmidt et al, 2003). The stable benign monoclonal marking provides an important contrast to the unstable monoclonal lymphoproliferation seen in the X-SCID studies.

2.1.5.2 Phase I trial of gene transfer to CD34+ cells from the bone marrow CD34+ cells from ADA-deficient SCID infants and children (2001-2009)

A Phase I trial of gene therapy for ADA-deficient SCID was performed by investigators at Childrens Hospital Los Angeles and the NIH Clinical Center (FDA BB IND# 8556) in a total of ten (10) subjects. This trial compared two slightly different γ-retroviral vectors, MND-ADA and GCsap-ADA, with half of the CD34+ cells in each patient getting one vector and the other half of their Cd34+ cells getting the other vector. The study was performed in two stages: 1. Four subjects

underwent the procedure in 2001-2002 and remained on PEG-ADA and did not receive busulfan chemotherapy and 2. Six subjects underwent the procedure in 2005-2009 and had PEG-ADA withdrawn and received non-myeloablative marrow conditioning with busulfan. Patient demographics, results of CD34+ transduction, and peripheral blood mononuclear cell marking are shown (see Table 3, below).

First stage without busulfan and on PEG-ADA:

In the first stage, the two younger subjects (202N and 204C at 4 years of age) had sustained gene marking, albeit at a low level, whereas the two older subjects (201C at 15 and 203N at 20 years of age, respectively) did not have detectable long-term marking. This finding suggests that younger subjects may be more likely to derive benefit from this approach, either due to better thymic function or a greater content of transducible stem cells in their bone marrow. There has not been any significant effect on immune reconstitution in these four subjects, and the patients have remained on PEG-ADA since participating in the study. Patient toxicity for these patients who did not receive busulfan and continued on PEG-ADA has been non-serious (class 1 or 2, primarily due to anemia from bone marrow harvest), and there has not been evidence of either lymphoproliferation or the presence of RCR. The subjects were treated while remaining on PEG-ADA enzyme replacement therapy and they did not receive pre-transplant conditioning chemotherapy.

It has now been shown in multiple studies (including our own described below) that it is necessary to "make space" in the marrow of patients undergoing autologous BMT with gene transfer for ADA-deficient SCID to appreciate significant engraftment of the gene-corrected cells. The initial experiences from Milan (n=10) and London (n=1) have been published (Aiuti 2002, Aiuti, 2007; Aiuti, 2009; Gaspar, 2009) documenting clinical benefit to subjects pre-treated with a non-myeloablative dosage of the chemotherapy agent busulfan prior to reinfusion of gene-modified CD34+ cells. Subsequent clinical trials of gene therapy for Chronic Granulomatous Disease, X-adrenoleukodystrophy, and beta-thalassemia have all used busulfan marrow conditioning to enhance engraftment of gene-modified stem cells (Ott, 2006; Cartier, 2009; Kaiser, 2009). ADA deficiency is a systemic metabolic disease, so engraftment of a larger amount of gene-corrected stem cells may be needed than in X-linked SCID, where pathology is confined to the immune system and a profound selective advantage exists for lymphocytes that have been corrected to express the common cytokine receptor gamma protein. Because of these considerations, we amended our clinical trial protocol to include the pre-administration of a non-myeloablative dosage of busulfan and this approach then comprised a second stage of the trial.

Second stage with busulfan and with PEG-ADA withdrawn:

We adapted this type of approach (marrow cytoreduction with Busulfan in patients off PEG-ADA) in an amendment to the original CHLA/NIH research protocol (approved 2005) which led to the second stage of the trial. We have enrolled **6** ADA-deficient SCID infant subjects under this amended protocol, between 2005 – 2009 (Table 3).

Safety:

Subject 302 had prolonged pancytopenia after administration of busulfan and administration of gene-modified CD34+ cells, persisting even after subsequent infusion of the non-processed "back-up" autologous marrow. She was subsequently found to have trisomy 8 mosaicism pre-existing in her marrow, which is known to lead to marrow dysfunction (Engel et al, *Blood* 109:503-6 2007). She had a successful unrelated donor HSCT and has been well. The protocol was amended to require demonstration of normal cytogenetics in blood or marrow cells as an eligibility criterion.

Subject 304 developed an infection at 4 months after treatment, and she was put back on PEG-ADA at the clinician's decision and she has continued to receive PEG-ADA since that time (2007). She also has several other medical problems, including obesity, type II diabetes, hepatic steatosis, reactive airway disease, sensorineural hearing loss, and developmental delay. She continues to have gene-containing and ADA-expressing PBMC, belying the putative blunting of selective advantage for ADA gene corrected cells by ERT.

Subject 306N developed an adenoviral infection at 5-6 months after transplant and was put back on PEG-ADA. ERT was stopped again in March 2011 and he is currently being followed at the NIH.

Table 3. Clinical Trials of Gene Therapy for ADA-Deficient SCID (As of June 2011)

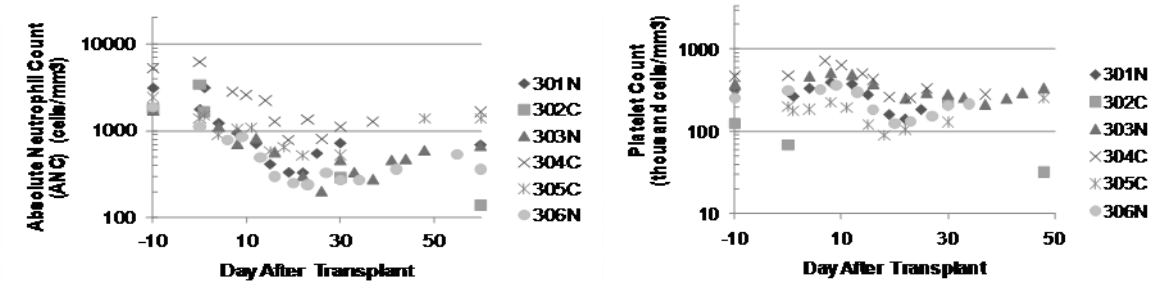
[redacted]	Gender	PEG-ADA	Busulfan mg/m^2	CD34+ Cells/kg	Long-Term Gene Marking	PBMC ADA Activity (nl >58 U)	Length of Follow-up (ca. 6/21/11)
usulfan, remain on PEG-ADA							
	F	not withdrawn	None	6.6 x 10^5	No	< 5 U	removed
	F	not withdrawn	None	9.75 x 10^6	Yes	< 5 U	10 yr
	F	not withdrawn	None	1.1 x 10^6	No	< 5 U	removed
	M	not withdrawn	None	1.88 x 10^6	Yes	< 5 U	9.5 yr
ulfan administered, PEG-ADA withdrawn							
	M	withdrawn	75	4.77 x 10^6	Yes	30-60 U	4.5 yr
	F	withdrawn/ restarted	75	1.5 x 10^5	Non-engraft Trisomy 8**	n.e. S/P MUD BMT	N/A*
	F	withdrawn	75	1.9 x 10^6	Yes	10-30 U	4 yr
	F	withdrawn/ restarted	64.6	1.6 x 10^6	Yes	50-110 U	3.5 yr
	M	withdrawn	90	9.75 x 10^6	Yes	60-120 U	2.7 yr
	M	Withdrawn/ restarted	90	9.5 x 10^6	Yes	10-20 U	2.2 yr

* (C= CHLA, N=NIH)

** 302C had trisomy 8 mosaicism and failed gene therapy (Engel, BC *et al.* Blood, 2007)

Besides the expected myelosuppressive effects of busulfan (moderate neutropenia with nadir ANC's of >200-300/ul^3 and mild thrombocytopenia with nadir platelet counts of >100,000/ul^3) (see Figure 2), there were grade I-II elevations of AST and ALT in most subjects (2-5x ULN at approximately 40 days after stopping PEG-ADA ERT and 30 days after administration of busulfan). There were no discernible clinical toxicities from the administration of busulfan (no seizures, N+V, diarrhea, mucositis, anorexia, or hair loss), with dilantin and ondansterone given prophylactically.

Figure 2. Representation of the myelosupressive effects of busulfan.



The kinetics of myelosuppression are different in this setting using only a single non-myeloablative dose of busulfan, compared to a typical conditioning regimen for allogeneic HSCT that combines busulfan with other drugs such as cytoxan or fludarabine. In a typical allogeneic transplant with multi-drug conditioning over the course of 5-7 days, neutropenia may be severe already by the day of transplant and generally recovers 2-3 weeks later. In this current study, we have observed slow progressive decline in neutrophils and platelet counts after the single dose of busulfan, with moderate neutropenia (ANC<500) being reached by 10-14 days. Cell counts reach nadirs 25-35 days after busulfan with relatively slow recovery of neutrophils for up to 3 months. No subject has had later problems with neutropenia or bacterial infections. A possible explanation for the different kinetics is that busulfan is uniquely active for the elimination of quiescent pluripotent stem cells to make space for donor engraftment, whereas most other chemotherapeutic agents are active against cycling progenitor cells and may lead to quicker cut-off of the supply of peripheral blood neutrophils and platelets.

Dr. Candotti and his group have identified a high frequency of abnormalities in the myeloid cells in ADA-deficient SCID patients, including spontaneous moderate neutropenia, unusual sensitivity to antibiotics and multiple cytological abnormalities, such as hyper-segmented nuclei (Sokolic, 2011). Inherent deficiencies of myelopoiesis in ADA-SCID may explain the slower recovery of neutrophils observed.

Clonality of vector integrants has been monitored at the National Gene Vector Biorepository using LAM-PCR; no evidence of clonal dominance or expansion has been observed.

Efficacy:

Subjects 301, 303, and 305 remain well with laboratory evidence of immune reconstitution without PEG-ADA therapy over 52, 48, and 32 months after gene therapy (as of June, 2011).

The gene marking in subjects 301, 303, 304, and 305 has been in the range of 1-10% in the PBMC fraction, with overall higher marking with the MND-ADA vector than the GCsap-M-ADA vector. All of the 5 evaluable subjects show the production of ADA enzyme activity in their PBMC (see Figure 3). This Phase I study is closed to accrual after enrolling the proposed total of 10 subjects, all of whom have been enrolled in a long-term follow-up study at UCLA or NIH to complete another 13 years of follow-up. These results are encouraging in terms of safety and efficacy. A Phase II trial was developed under the same IND (BB# 8556) to extend the studies to an additional cohort.

Figure 3. Gene Marking in +Bu/-ERT Subjects.

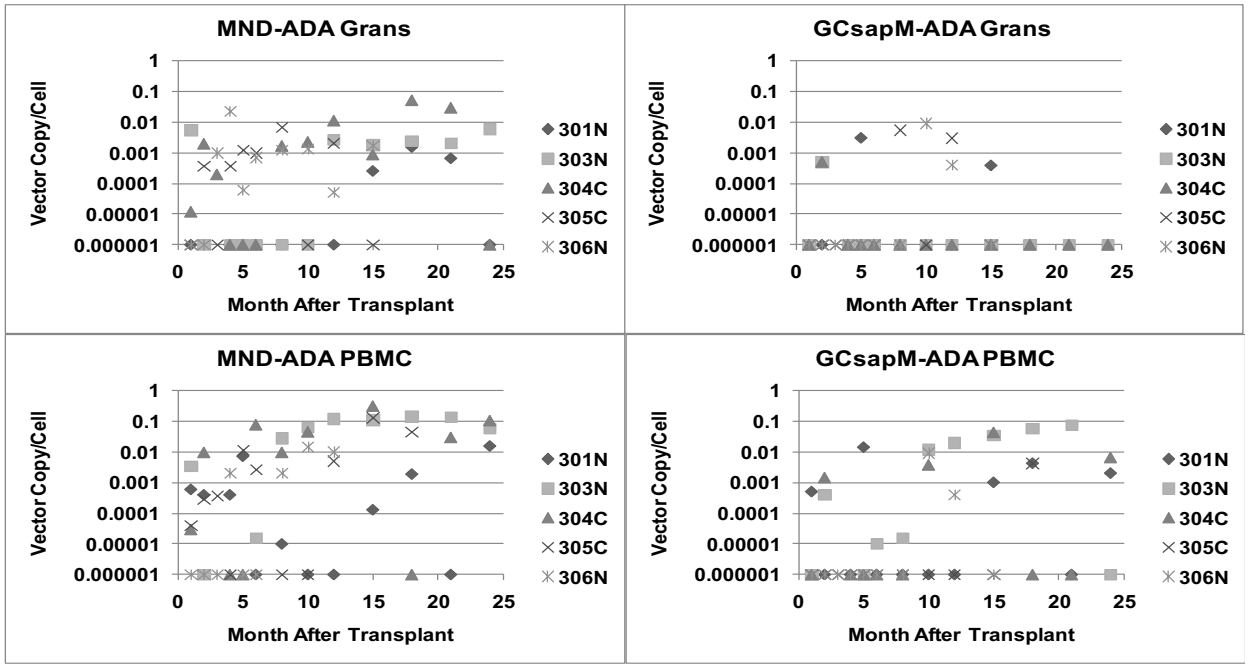
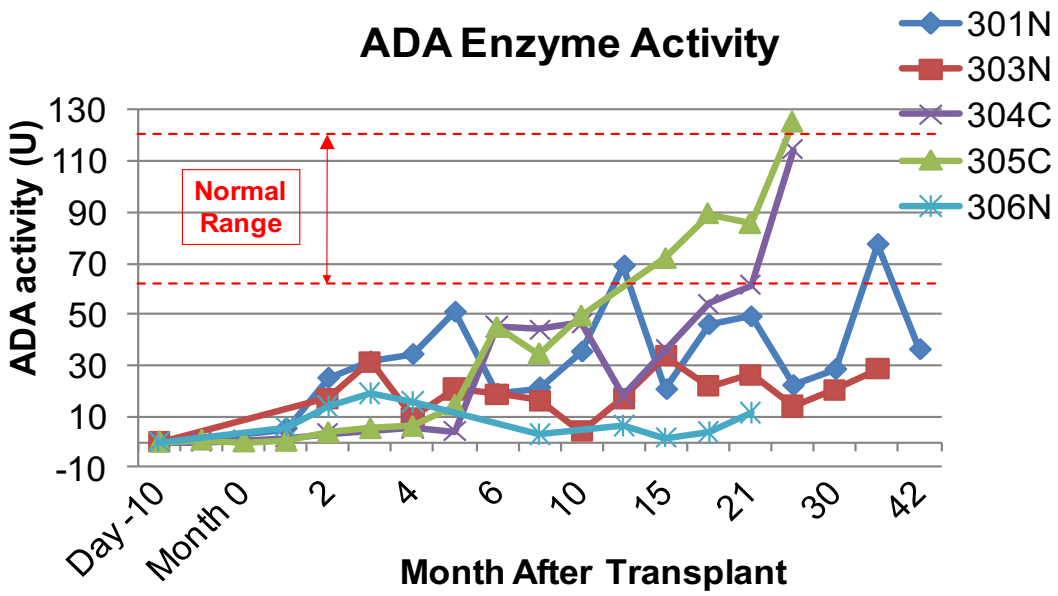


Figure 4. ADA Enzymatic Activity



Results of this clinical trial were published (Candotti et al, Blood 2012)

2.1.5.3 Phase II trial of gene transfer to CD34+ cells from the bone marrow CD34+ cells from ADA-deficient SCID infants and children (2009-2014)

A Phase II clinical trial evaluating the efficacy of a single γ-retroviral vector (MND-ADA) in a total of 10 ADA-deficient SCID subjects was performed at UCLA and the NIH, and funded by an FDA Oprhan Product Development Grant (1 R01 FD003005). In addition to only using a single vector (MND-ADA), the Phase II trial had two modifications from the prior Phase I study. An amendment to this Phase II Trial was approved in June 2009 (1) to lower the minimum age limit for enrollment from 6 months to 3.5 months of age and (2) to consolidate the administration of the busulfan to a single dose (90 mg/m^2, ~ 4 mg/kg). In March 2010, we submitted an amendment to further lower the age of entry to 1.0 month and this was accepted by the FDA, the IRB at UCLA and NIH and the NHBLI DSMB. The lower limit of 6 months old was imposed in the Phase I trial in 2004 under FDA guidance following the first two cases of T lymphoproliferative disease in the XSCID study. However, subsequent cases were in subjects older than 6 months at the time of treatment, and thus, young age does not appear to be a significant risk factor. Opening the study to younger subjects may allow them to be treated prior to acquisition of infections.

Administration of IV busulfan once daily has been shown to have essentially identical effects as giving it divided into 4 doses q 6 hours as was done with the oral preparation (e.g. Almog, BBMT, 2011). The single dosing schedule will allow an easier treatment regimen and will allow a single set of blood samples to be taken for pK determination, rather than two complete sets.

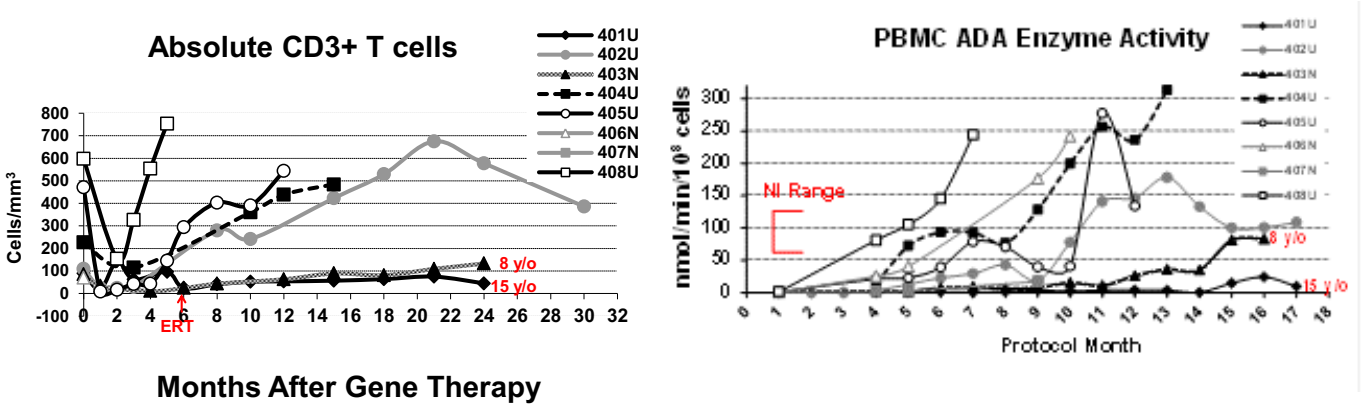
Ten subjects were enrolled and have undergone the procedure between 2009-2014, with seven subjects at Mattel Children's Hospital at UCLA, and three at the NIH (Table 4).

Table 4. Enrolled Subjects since 2009 to Phase II Study.

[redacted]	Age at Entry	Gender	PEG-ADA	Busulfan mg/ m^2	CD34+ Cells/kg	ALC at 6 Months	PBMC ADA Activity (nl>58U)	Length of Follow-Up (Oct. 2016)
ministered, PEG-ADA withdrawn								
	15 yr	M	withdrawn/ restarted	90	$0.6x10^6$	96	< 5 U	72 mo
	4 mo	F	withdrawn	90	$1.7x10^6$	480	120-180 U	72 mo
	8 yr	M	withdrawn	90	$1.4x10^6$	70	10-20 U	72 mo
	3 mo	F	withdrawn	90	$7.1x10^6$	699	70-120 U	72 mo
	8 mo	M	withdrawn	90	7.6×10^6	306	39.8	60 mo
	13 mo	F	withdrawn	90	8.0×10^6	120	176.6	60 mo
	14 mo	M	Withdrawn	90	5.6×10^6	50	16.9	60 mo
	3 mo	F	withdrawn	90	6.8×10^6	1050	272	60 mo
	19 mo	F	withdrawn	90	2.9×10^6	160	18.9	42 mo
	3 mo	F	withdrawn	90	8.4×10^6	2200	527.8	42 mo

There have been no severe adverse events in these subjects, besides busulfan induced transient moderate neutropenia and mild thrombocytopenia. PBMC ADA enzyme activity and absolute lymphocyte counts have remained low in subjects 401 and 403, who were the two oldest subjects in this series (15 and 8 y/o, resp.) (see Figure 5). Subject 401 was re-started on PEG-ADA ERT after 6 months due to absence of efficacy. Subjects 402 and 404 have had recovery of lymphocytes (ALC> 500 by 6 months) and PBMC ADA enzyme activity in the normal range. Subject 405U was treated only 5 months ago and 406N one and they are not yet evaluable.

Figure 5. Levels of absolute lymphocyte counts and PBMC ADA enzyme activity.



In total, our study and those from Milan and London confirm the potential efficacy from autologous transplantation using γ-retroviral-mediated ADA gene transfer to bone marrow CD34+ cells with non-myeloablative cytoreductive conditioning. There appears to be a difference in responses to this procedure between younger (< 2 years) and older (> 5 years) subjects, when combining results from the Phase I and Phase II studies.

Table 5. Summary of ADA-Deficient SCID Patients.

Summary of ADA-Deficient SCID Patients
Retroviral Vectors, Myeloreductive Conditioning
–Milan/London/CHLA-NHGRI, NIH-UCLA

Center	# Pts	F/U (yrs)[1]	Off Enzyme	Survival	DFS[2]
Milan	17	0.9 – 10.5	14/17	100%	82.4%
London	8	0.5 – 7.5	4/8	100%	50%
CHLA-NHGRI UCLA-NHGRI	6 8	2– 5 0.1-2	3/6 7/8	100% 100%	50% 87.5%
TOTAL	**39**	**0.3 – 9.0**	**28/39**	**100%**	**72%**

[1] As of April 2012
[2] DFS ≡ Alive without BMT or PEG-ADA re-start

Data: Courtesy HR Gaspar (London) and Alessandro Aiuti (Milan)

2.1.6 Summary of Relevant Clinical Studies

2.1.6.1 Complications from Vector Integration with γ-Retroviral Vectors

Stable and persistent gene correction of hematopoietic stem cells has required the use of gene delivery vectors that can covalently integrate the transferred gene into cellular chromosomal DNA so that the sequences may be replicated and passed on to the billions of progeny blood cells that derive from the stem cells. The relatively random insertion of γ-retroviral vectors poses risks of "insertional oncogenesis" (**IO**), in which vectors integrated in the vicinity of critical cellular genes may alter their expression; changes in the expression of genes that affect cell proliferation, survival or other biological properties may initiate a cascade of cell proliferation, initially manifest as a monoclonal expansion which may ultimately culminate in frank malignant transformation. Complications from vector-mediated IO have occurred in several clinical trials of gene transfer using γ-retroviral, and more recently lentiviral vectors.

After immune reconstitution in 9 of 10 subjects participating in an **X-SCID** gene transfer study in Paris, France four of these subjects developed leukemia ≥ 2½ years after receiving the gene-

transduced CD34+ cells (Cavazzana-Calvo M et al 2000; Hacein-Bey-Abina S et al 2002, Hacein-Bey-Abina, S et al., 2003A; Hacein-Bey-Abina, S et al., 2003B; Hacein-Bey-Abina S et al., 2008). In a similar trial of gene therapy for X-SCID performed in London, England, one of 10 subjects also developed T lymphoproliferation (Howe J et al, 2008). One of these five patients who developed T lymphoproliferative disease (LPD) has died and the other four are in leukemia remission and continue to have restored immunity from the gene therapy. For three of the subjects with leukemia, the proviral insertion sites have been mapped within or near (3 kb) of the LMO-2 gene (Hacein-Bey-Abina, S., et al, 2003), a human proto-oncogene associated with T-cell leukemia (Neale et al. 1997; Mead et al. 2001; Rabbitts, T. H. et al. 1998). In a fourth case, two integrations sites were described: LMO-2 and BMI1, which is also a proto-oncogene. In a fifth subject, vector had integrated near a third proto-oncogene, CCND2 (Hacein-Bey-Abina S et al., 2008). These are the first reported cases of leukemia developing in subjects participating in a clinical study of γ-retroviral-mediated gene transfer to CD34+ cells. The probability of leukemia developing in other participants of the French trial, or in participants of other trials, is not known.

Two young men with **X-linked Chronic Granulomatous Disease** were treated by γ-retroviral vector-mediated transfer of the relevant oxidase cDNA to G-CSF mobilized peripheral blood stem cells (Ott, 2006). The vector had viral long-terminal repeats (LTR) from the murine Spleen Focus Forming Virus, which has very potent enhancer/promoter activity in myeloid cells to drive high level expression of the transferred gene. Both subjects had oligoclonal expansion of stem/progenitor cells containing vector integrants near a few cellular genes involved in myeloid cell proliferation, which progressed to myelodysplasia.

In Germany, 7 of 10 children receiving γ-retroviral-mediated gene transfer for WAS, developed an acute T-cell leukemia (Braun, CJ et al., 2014).

Of note, to date (October 2016), at least 40 **ADA-deficient SCID** patients have undergone these protocols using γ-retroviral vectors with intact LTR in Milan, London and in our trials at CHLA/NIH and UCLA/NIH. No cases of T LPD or other adverse consequences of vector insertion have occurred in any of the ADA subjects, in sharp contrast to the X-SCID results (Aiuti, 2007).

2.1.6.2 Lentiviral Vectors May be More Effective and Safer

Lentiviral vectors were developed in the mid 1990's from components of the HIV-1 lentivirus. Lentiviral vectors have been found to have several attributes that make them potentially more effective and safe than γ-retroviral vectors (Naldini, et al, 1996; Zufferey, et al, 1997; Zufferey, et al,1998). Lentiviruses, but not retroviruses, can transduce non-dividing cells, a property of the most primitive and long-lived HSC. Lentiviral vectors are more effective than γ-retroviral vectors when compared using multiple surrogate assays for human HSC, including *in vivo* growth in NOD/SCID mice and eLTCIC assays of quiescent CD34+/CD38- cells (Miyoshi, et al, 1999; Case, et al,1999). In our studies, only the lentiviral vector could transduce CD34+/CD38- cells, a phenotype that greatly enriches for quiescent cells of higher proliferative and differentiation potential; MLV vectors showed essentially no transduction of CD34+/CD38- cells. Lentiviral vectors can perform effective transduction during just 1-2 days of culture (in contrast to the 3-5 days needed using γ-retroviral vectors); this shorter time of culture has been clearly proven to increase the survival of pluripotent stem cells (Mazurier, 2004; Kennedy, 2009). Transduction by

lentiviral vectors could be achieved even with a single "hit" on the first day of cell isolation. Transduction could also be achieved in the absence of recombinant cytokines, although the efficiency was approximately 2-fold less than in the presence of factors such as IL-3/IL-6/SCF. Ideally, this will lead to more effective transduction of quiescent HSC with preservation of stem cell function.

Thus, lentiviral vectors show greatly improved ability to transduce human HSC which may lead to increased gene modification *in vivo*. More effective gene transfer to long-term reconstituting pluripotent hematopoietic stem cells may lead to more robust and rapid immune reconstitution than with using γ-retroviral vectors.

Additionally, lentiviral vectors configured to lack strong the strong enhancer elements of γ-retroviral long-terminal repeats, present in typical γ-retroviral vectors, have been shown to have lower risks for causing insertional oncogenesis in a number of *in vitro* and *in vivo* models. This may translate into a lower risk for causing leukoproliferative disorders in gene therapy subjects, However, one subject of a clinical trial using a lentiviral vector for gene therapy of **beta-thalassemia** had monoclonal expansion from a stem/progenitor cell in which the integrated vector altered the expression of the HMGA2 gene, although there have been no hematological abnormalities observed to date (in fact, the subject has had a significant clinical benefit). The vector used contained elements from the β-globin gene cluster locus control region, which has strong enhancer activity, which may play a role in over-expression of the HMGA2 gene. But, the mechanism for clonal expansion may be due to a novel gene disruption event, with the vector integrated into the 3rd intron of the HMGA2 gene leading to aberrant splicing, production of a truncated HMGA2 gene transcript and protein, with loss of 3' regulatory sequences involved in expression repression by a microRNA (Kaiser, 2009).

In contrast, a recently reported clinical trial using a lentiviral vector for gene therapy of **X-adrenoleukodystrophy** has not encountered any adverse vector insertional effects, despite high level of engraftment of gene-transduced HSC (Cartier, 2009). There are pre-clinical data which indicate that lentiviral vectors *per se* have moderately lower risks for causing IO, compared to γ-retroviral vectors (Montini, 2009). Additionally, lentiviral vectors that have "self-inactivated" (SIN) LTR with deletion of their enhancers and instead use cellular promoters lacking strong enhancers (as in the EFS-ADA vector to be used in this Protocol), have essentially undetectable IO activity in current assays (Modlich, 2009).

Lentiviral vectors have entered the clinic in a trial with HIV-1-infected subjects (performed by investigators from VirXSys Corp. and the University of Pennsylvania) (Levine, 2006). This study targeted mature T cells collected by apheresis for *ex vivo* transduction with a lentiviral vector encoding antisense RNA to HIV-1 *env* sequences. There was relatively effective gene delivery to the T cells, with ongoing detection of gene-modified T cells for more than one year in two of the subjects. Follow-up over two years did not detect adverse clinical effects.

Lentiviral vectors to transduce CD34+ HSC are only recently entering human clinical trials. One trial to use a lentiviral vector to introduce a shRNA into G-CSF mobilized PBSC to down-regulate the HIV-1 co-receptor CCR5 yielded only very low levels of gene-containing peripheral blood cells (Zaia, 2009). Importantly, in a recently reported clinical trial of gene therapy for X-linked

adrenoleukodystrophy, the first to use a lentiviral vector to transduce CD34+ PBSC and which used full cytoablative conditioning, 10-20% gene marking was obtained in all blood cell lineages both subjects for at least one year (Cartier, 2009). One subject in a clinical trial in France using a lentiviral vector carrying a human beta-globin expression cassette attained up to 10% gene marking and clinical improvement to the point of becoming transfusion-independent (Cavazzana-Calvo, 2010).

As of October 2016, 20 patients have been treated at UCLA with OTL-101, and the trial is closed to enrollment. At last analysis cut-off date (31 March 2016), 18 evaluable patients have 100% overall survival and 100% event-free survival (defined as not returning to ERT or requiring a rescue HSCT). Additionally, 15 evaluable patients (of 18 patients total, including compassionate use patients) have been treated with the related product at Great Ormond Street Hospital (GOSH)/University College London (UCL) with 100% overall survival and 93.3% event free survival. At UCLA all of the patients with sufficient follow-up have also stopped immunoglobulin replacement therapy (IgG RT) and all but one has stopped at GOSH/UCL.

2.1.7 Description of the Study Agent(s)/Intervention(s)

The EFS-ADA lentiviral vector: The EFS-ADA lentiviral vector (see Figure 6) was constructed in the research laboratory of Adrian Thrasher at University College London, United Kingdom. **The key goals of this vector are to attain efficient ADA gene transfer with sufficient expression with highest safety.** The vector backbone is the pCCL vector, an HIV-1 lentiviral-based, self-inactivating (SIN) vector, described by Zufferey, et al (1998)**.** SIN vectors have the enhancer and promoter elements deleted from the long-terminal repeat (LTR), eliminating the most significant vector factor in causing insertional oncogenesis (IO); vector of this design may have lower risks for causing IO, improving the outcomes for patients. Instead of using the vector's LTR enhancer/promoter to drive transcription of the ADA transgene, a codon-optimized human ADA cDNA was cloned downstream of the "short" human elongation factor alpha-1 promoter (EFS), which lacks the intron and enhancers of the larger EF1-α sequences used in many expression plasmids. The "EFS" promoter has been shown to direct high level transcription of reporter genes in murine hematopoietic cells and to have significantly reduced *trans*-activation potential compared to γ-retroviral LTR (Zychlinski, et al, 2008). A WPRE element with the open-reading frame eliminated is present to boost titer and gene expression.

Figure 6. Lentiviral Vector: EFS-ADA

huADA cDNA = human ADA cDNA; EFS = elongation factor 1α short (see text for description); WPRE = woodchuck hepatitis post-transcriptional regulatory element.

The EFS-ADA lentiviral vector is packaged by co-transfection of its encoding plasmid into human 293T cells along with a plasmid encoding the HIV-1 virion and enzymatic polyproteins GAG and POL, another plasmid encoding the HIV-1 REV protein to facilitate vector RNA genome nuclear export, and the VSV-G protein to pseudotype the vector for stability to allow vector concentration and high efficiency transduction of human CD34+ cells. The concentrated GMP-grade EFS-ADA vector will be produced at the Indiana University Vector Production Facility under contract through the NHLBI Gene Therapy Resource Program. It will be fully characterized to meet Release Criteria agreed upon with FDA CBER, including purity (microbial, absence of replication competent lentivirus), potency (titer, transduction of human bone marrow CD34+ cells, expression of ADA enzymatic activity) and identity (sequence of vector provirus). Lentiviral vectors produced by this method of transient transfection have been used in several clinical trials without incident.

2.1.8 Summary of Pre-Clinical Studies with Study Agent/Intervention

2.1.8.1 Evaluation of ADA Lentiviral Vectors

a. Human hematopoietic and lymphoid cell lines:

ADA gene transfer and expression by a series of lentiviral vectors was studied in a panel of human cell lines (HT29 colon carcinoma, K562 erythroleukemia, CEM T lymphoblastic, and HTVL-1 immortalized ADA-deficient SCID T cells). The transduced cells were assayed to quantify the vector copies (VC)/cell, using qPCR, and the ADA enzymatic activity, using a colorimetric biochemical assay. Expressed ADA enzymatic activity per vector copy was then calculated. The EFS-ADA lentiviral vector (here called CCL-c-sEF1a-hADA – far right bars) expressed ADA enzyme activity well (~1-2 U/VC). It yields a single band on Southern Blot (not shown).

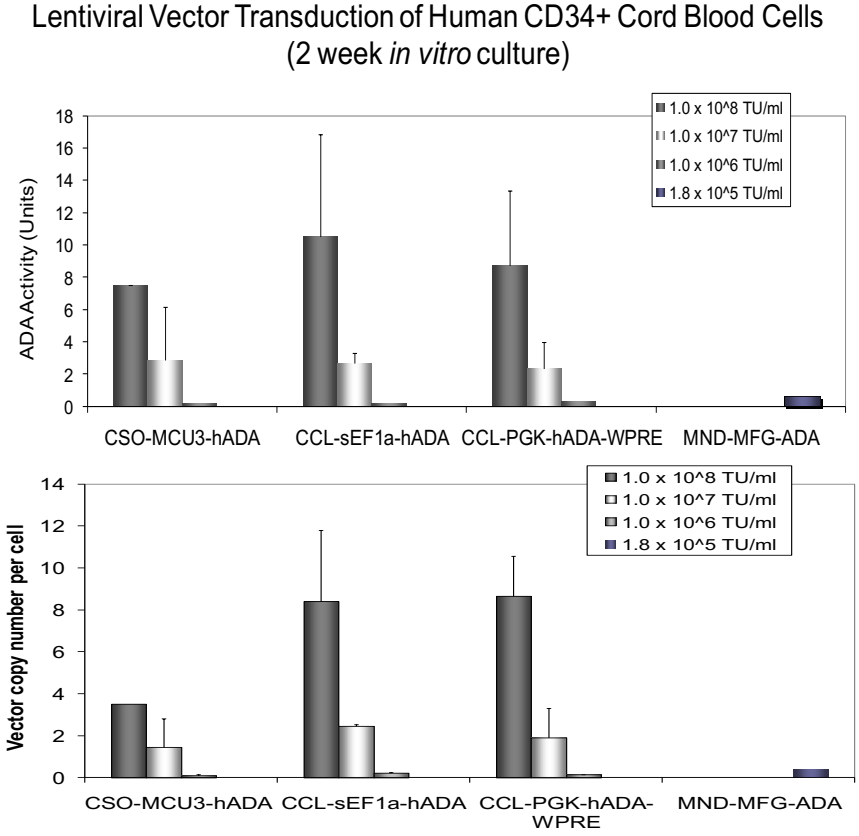
b. Human CD34+ cord blood cells *in vitro*:

Studies of these lentiviral vectors have also been performed using human CD34+ cells isolated from normal umbilical cord blood (see Figure 7). The CD34+ cells were transduced under the culture conditions to be used for this clinical trial (see Section 5.1), testing a range of vector concentrations (10^6, 10^7 and 10^8 TU/ml). Additionally, the MMA-hADA (MND-MFG-ADA) γ-retroviral vector that is currently being used in our ongoing clinical trial was also compared. The clinical MND-MFG-ADA vector supernatant produced from a stable PG13 cell clone has a titer of approximately $1.8x10^5$ TU/ml. The transduced CD34+ cells were grown in culture for two weeks,

and then cell samples assayed to quantify the vector copies/cell, using qPCR, and the ADA enzymatic activity, using a colorimetric biochemical assay. Expressed ADA enzymatic activity per vector copy was then calculated.

These studies show that transduction, as measured by vector copy per cell, is directly related to the concentration of vector during transduction, with 10^7 TU/ml leading to approximately 2 copies/cell for each of the lentiviral vectors. In contrast, the MMA-hADA γ-retroviral vector currently being used in our clinical trial, has a lower titer ($1.8x10^5$ TU/ml) and led to only ~0.2 copies/cell. Expression of the transferred ADA cDNA was at a similar level by the EFS-ADA lentiviral vector to be used in this clinical trial, the MMA-hADA γ-retroviral vector currently in trial and a lentiviral vector with the MND LTR (pCSO-re-MCU3-hADA), (~1-2 U/vector copy).

Figure 7. ADA activity and vector copy number results of human CD34+ cord blood cells trasduced with lentiviral vectors.



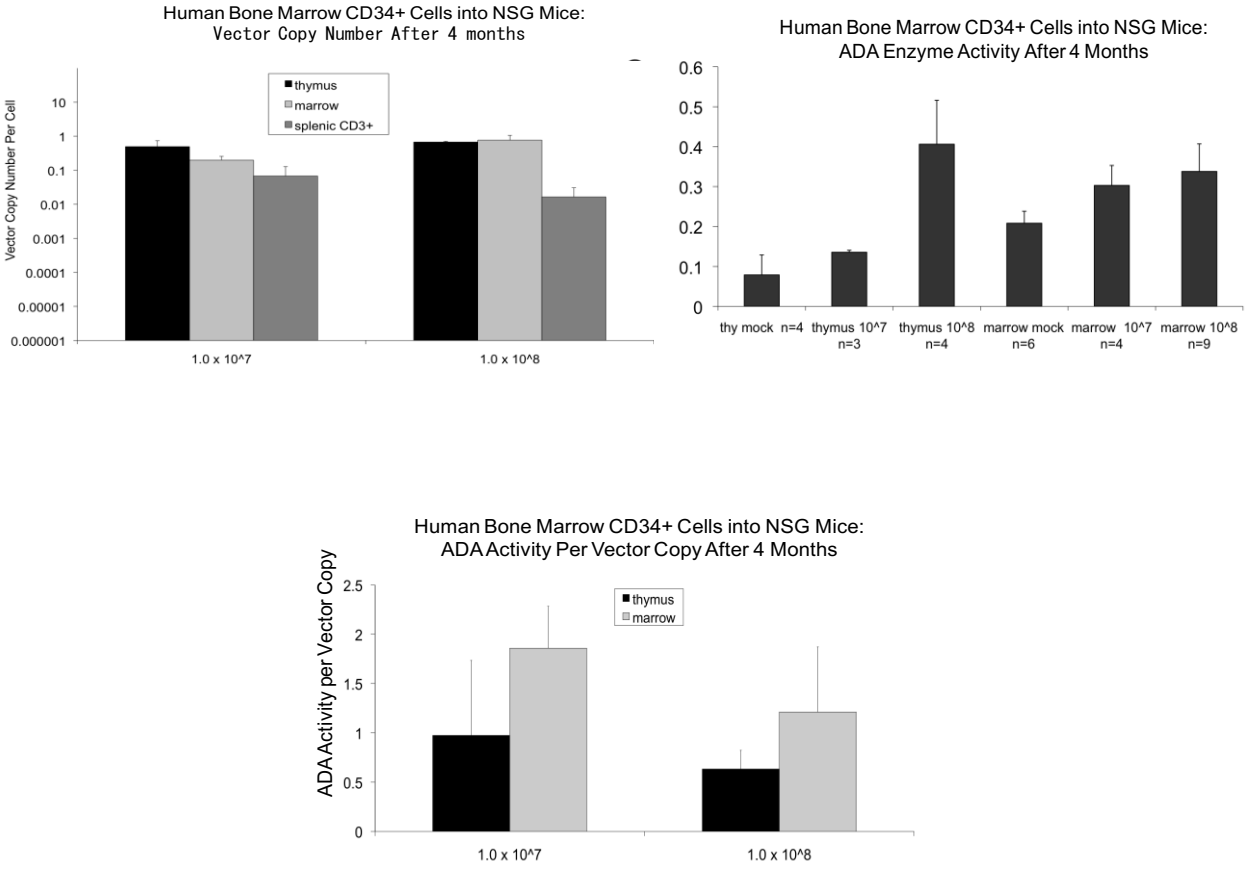
2.1.8.2 Transduction of Human CD34+ cells with EFS-ADA

a. Human CD34+ bone marrow cells in vivo:

Human CD34+ cells from normal donor bone marrow were transduced with the EFS-ADA lentiviral vector and transplanted by intravenous injection into neonatal NOD/SCID/γc(null) (NSG) mice. After four months, the mice were euthanized and their spleens (Spl), bone marrow (BM), and thymuses (Thy) were harvested. Portions of each organ were analyzed by flow cytometry and used to make lysates for ADA enzyme assay and qPCR measurement of vector copies (VC)

/cell and other portions were used to isolate specific human cell populations by immunomagnetic bead separate (CD3+ T cells from spleen, and CD45+ human leukocytes from the bone marrow) and these sub-populations were analyzed by FACS and extracted for ADA enzyme assay and qPCR for VCN.

Figure 8. Vector copy number and ADA activity results in human bone marrow CD34+ cells into NSG mice.



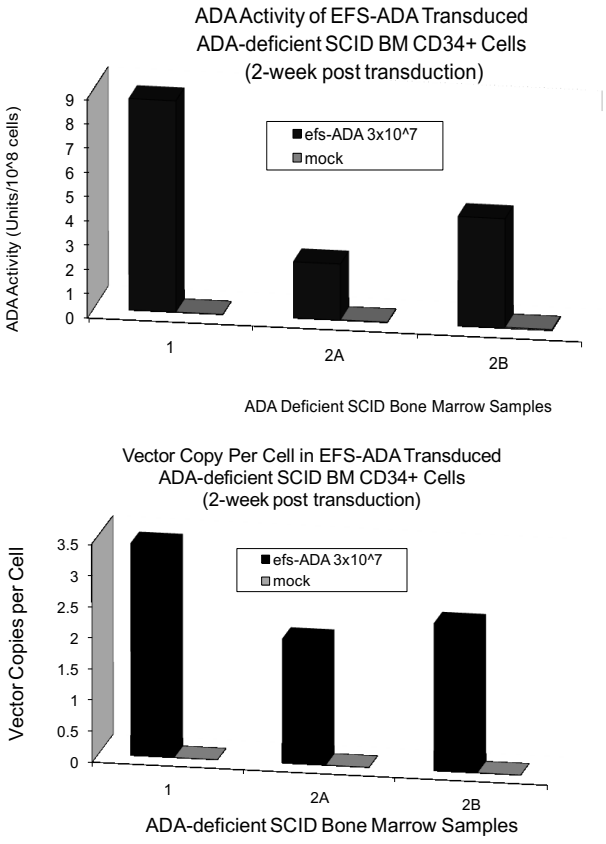
There was a dose-dependent relationship between lentiviral vector concentration during CD34+ cell transduction and both the ADA enzyme activity measured (above right) and the vector copy per cell (above, left). Human cells contained approximately 0.1-1 VC/cell and this yielded 1-3 fold net ADA enzyme activity compared to the endogenous activity in the mock-transduced cells (from normal, not ADA-deficient human donors). The ADA activity per vector copy number was in the range of 1-2 U/VC.

b. Human ADA-deficient SCID bone marrow *in vitro* and *in vivo*.

Small aliquots of bone marrow were obtained from two of the ADA-deficient SCID subjects enrolled in the γ-retroviral vector-mediated ADA gene transfer trial during marrow harvest (samples 1 and 2B) or during a diagnostic marrow aspiration (sample 2A) under UCLA IRB #09-04-012-01. CD34+ cells were isolated from the marrow samples and they were transduced with the EFS-ADA vector using the proposed clinical methods, with a final vector concentration in the cultures of 3 x 10e7 TU/ml. Following the single exposure to vector, a portion of the cells was injected into NSG mouse neonates and the remaining portion was expanded in short-term culture for two weeks and then assayed for ADA enzyme activity and vector copy numbers per cell.

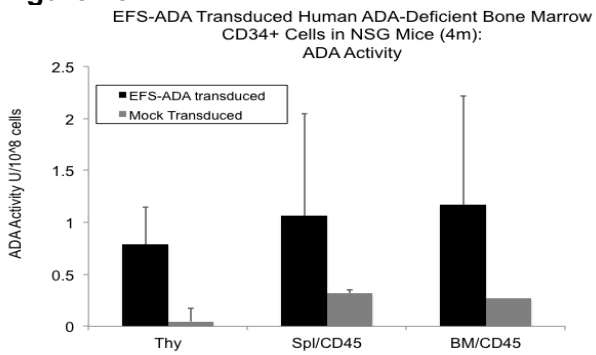
In the analysis of the aliquots that were grown *in vitro* for two weeks, ADA activity in the mock-transduced cells from the ADA-deficient donors was essentially undetected and was significantly increased in the transduced cells. Vector copy numbers per cell ranged from 2-3.5. The calculated ADA activity per vector copy was 1-2.5 U/VC.

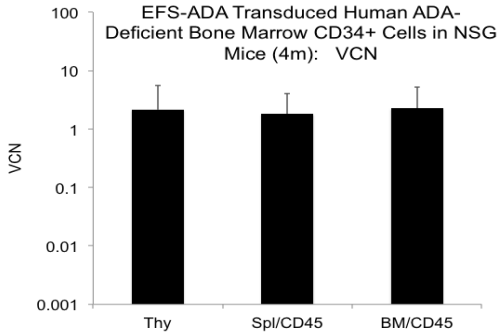
Figure 9



The NSG mice were analyzed four months after neonatal injection and analyzed as described above (see figures below). After 4 months, the EFS-ADA transduced cells human ADA-deficient cells isolated from the murine tissues had 3-10 fold more ADA enzyme activity than the mock transduced cells with 0.1-5.0 vector copies per cell, averaging 1-2 U/VC.

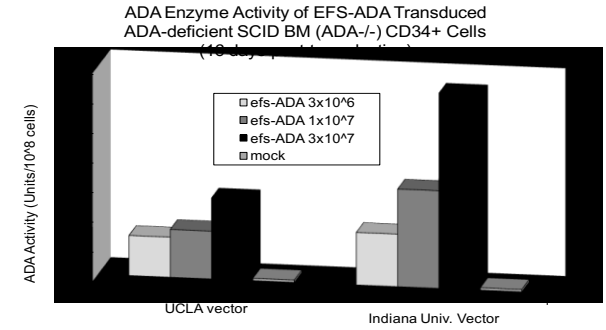
Figure 10

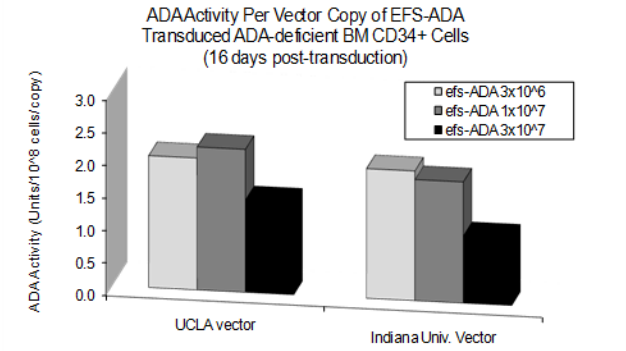


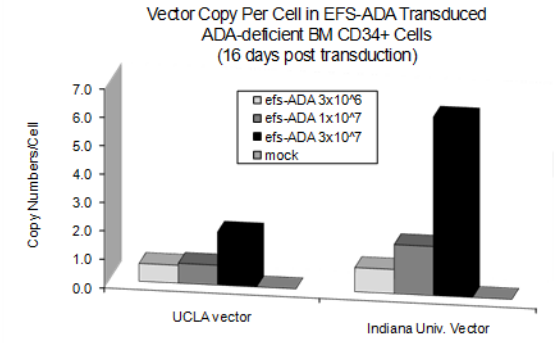


c. GMP-comparable batch of EFS-ADA manufactured at the IUVPF. In a separate study, a cryopreserved aliquot of CD34+ cells from sample 2B (above) was used to assess the activity of a GMP-comparable batch of EFS-ADA manufactured at the IUVPF. A sample of EFS-ADA vector manufactured at UCLA with less extensive purification was used in comparison and equivalent TU/ml of the two preparations were used, following the clinical transduction methods, based on titer determination at UCLA.

Figure 11







ADA activity increased proportionately to vector concentration above the low background of the mock-transduced ADA-deficient cells, although the rise was steeper with the IUVPF preparation. Similar dose-related effects on vector copy number were seen. The net ADA activity per vector copy was between 1-2 U/VC. These data indicate that the vector preparation from the IUVPF was more effective at transducing the human CD34+ cells, but ADA enzyme expression per vector copy was similar.

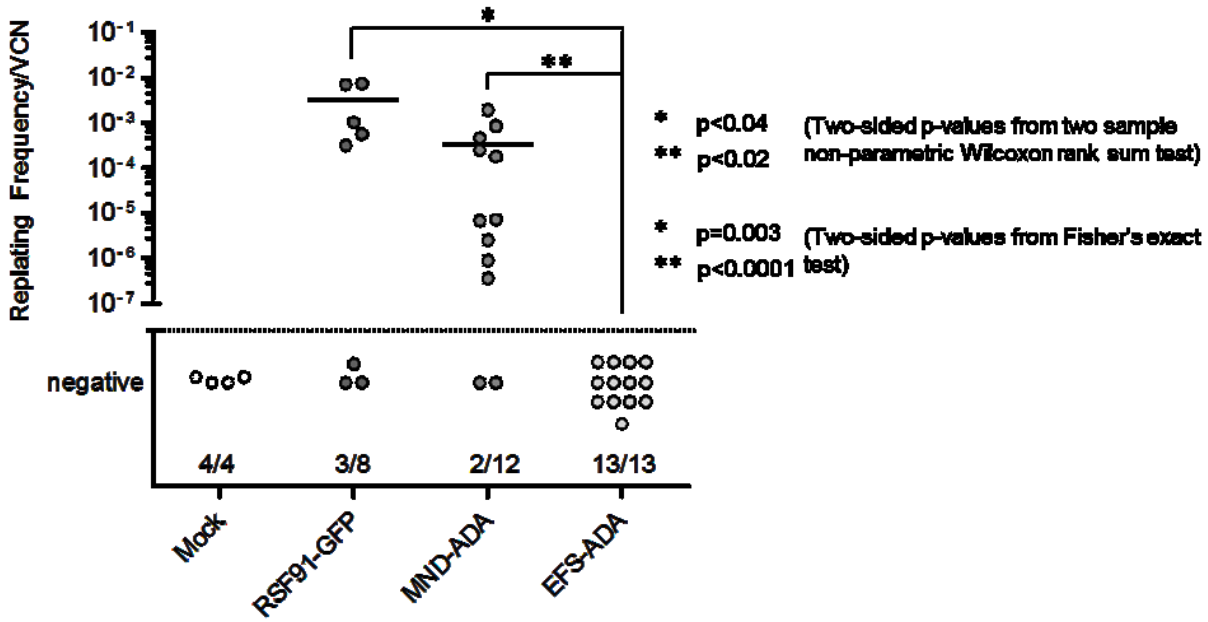
2.1.8.3 In vitro immortalization (IVIM) assay

To measure the insertional oncogenesis activity of the EFS-ADA vector, n vitro insertional mutagenesis (IVIM) assays were performed with using murine bone marrow depleted of cells

expressing mature lineage markers (Lin-) transduced by the EFS-ADA lentiviral vector, the SR91GFP positive control gamma-retroviral vector with intact SFFV LTRs previously shown to cause a high frequency of progenitor immortalization (Modlich. 2006), the MND-ADA γ-retroviral vector or mock-transduced. Cells were cultured for two weeks after transduction under conditions which lead to HSC depletion unless a vector integrant causes clonal immortalization. A sample of the cells was taken for VCN determination, and then cells were plated by limiting dilution at 100 cells/well and 1,000 cells/well. After 2-3 weeks, replating frequency was determined based on scoring wells with vigorous growth of cells.

The SF91GFP and MMA gamma-retroviral vectors produced abundant clones, with replating frequencies between 1x 10e4-10e2. The frequency of replating by marrow transduced with the EFS-ADA lentiviral vector was significantly lower than by the cells transduced with either of the gamma-retroviral vectors, with no colonies formed by the EFS-ADA-transduced cells across all 4 assays performed, similar to mock-transduced marrow.

Figure 12



In total, these data support the use of the EFS-ADA vector to achieve ADA expression in vivo in human cells derived from the transduced CD34+ cells. It can efficiently and reproducibly transfer and express the human ADA cDNA in human CD34+ cells from ADA-deficient SCID subjects.

2.2 Rationale

Because a small number of ADA-corrected T lymphocytes may have a selective survival advantage and lead to immune reconstitution, the current levels of gene transfer using γ-retroviral

vectors have been sufficient for clinical benefit in some subjects. **In this Protocol, with the improved gene transfer and engraftment of HSC that may be expected using a lentiviral vector, we anticipate a more marked clinical benefit as measured by higher gene marking and improved immune reconstitution status without PEG-ADA, which will form the Secondary Objectives of this study.** Pre-clinical studies suggest that the lentiviral vector configuration to be used (enhancer-deleted LTR and internal EFS promoter) has a significantly lower risk for causing insertional perturbations of cellular genes than the γ-retroviral vectors we have used before, although there are no human clinical data.

There have been no clinical trials to date utilizing lentiviral vectors for gene-correction of ADA-SCID. We will use the EFS-ADA lentiviral vector with the human ADA cDNA to transduce autologous CD34+ cells from the bone marrow of ADA-deficient SCID patients. We will assess safety (as primary end-point) and also evaluate the production of gene-containing peripheral blood cells, ADA enzyme levels in peripheral blood cells, and immune function (as secondary end-points) in twenty (**n=20**) subjects. This Phase I/II clinical trial will be conducted at Mattel Children's Hospital at UCLA and at the NIH. [A parallel study will be performed in the United Kingdom at Great Ormond Street Hospital (GOSH)/University College of London (UCL) using the same lentiviral vector and a similar clinical trial.] The study is open to infants and children diagnosed with ADA-deficient SCID who do not have a medically eligible, HLA-identical sibling donor for bone marrow transplantation. This trial will evaluate the safety (as primary endpoint) and efficacy (as secondary end-points) of a lentiviral vector (EFS-ADA).

We hypothesize that the EFS-ADA lentiviral vector will result in increased transduction efficiency of the CD34+ cells, compared to the γ-retroviral vectors used previously. We anticipate that this increased transduction will result in better engraftment of gene-corrected stem cells, which will in turn result in increased ADA gene marking in peripheral blood cells, more rapid and robust production of gene-corrected lymphocytes that express ADA, and a reconstituted immune system compared to the current standard of care, hematopoietic stem cell transplant (HSCT).

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

Withholding of PEG-ADA:

As part of the treatment protocol, subjects are withdrawn from their PEG-ADA (if they are on it) 30 days after receiving their gene-modified cells to select for the propagation of gene-modified cells after they have been re-infused. Withdrawal of PEG-ADA can have adverse effects on immunity, as well as on other organ systems. Once PEG-ADA is withdrawn, the numbers of circulating T, B, and NK lymphocytes will decline and may remain low for several months until the autologous transplant of gene-corrected CD34+ cells leads to immune reconstitution. It is possible that an infection of high severity may develop during this time and this could even be fatal if it does not respond to therapy. If immunity is severely decreased after discontinuation of enzyme replacement therapy and the subject experiences multiple or serious infections, then therapy may have to be resumed.

Other Risks of infection:

It is possible that, because of the trips that are required to participate in the trial, patients may get exposed to infections that they could have avoided by not traveling. This will primarily be a consideration during the follow-up after discharge from the hospital. These patients then will not be in isolation and therefore exposed to the potential pathogens found in the public venues they visit. For these patients, it is difficult to clearly assess whether or not traveling constitutes an additional risk, and its potential significance. For patients whose clinical condition is not judged stable enough to travel (that is, hospitalized or at home with clinical signs of infection), screening or follow-up visits will be rescheduled after the resolution of the clinical problem.

Busulfan toxicity:

Busulfan has been widely used for myeloablation in bone marrow transplantation and has a well-described toxicity profile. When Busulfan is used at high doses (16 mg/kg, which translates to approximately 40 mg/m^2 x 16 doses) along with cyclophosphamide, as administered prior to allogeneic bone marrow transplantation, significant toxicity including thrombocytopenia and neutropenia have occurred, Busulfan-related seizures and emesis may occur and are largely preventable with prophylactic medication. Rarely, veno-occlusive disease and pulmonary fibrosis (the latter two toxicities seen with Busulfan/cyclophosphamide combinations) have been described. However, the nature and extent of toxicity resulting from low-dose Busulfan (e.g. 4 mg/kg IV x 1) in a pediatric population are not known. A recent report in ten ADA-SCID children demonstrated evidence of neutropenia but suggested no limiting toxicity with 4 mg/kg (Aiuti, A et al., 2002, 2009). These complications of high dose busulfan in combination with other chemotherapy drugs has not been seen in any of the more than 25 ADA-deficient patients treated using low dose busulfan.

Busulfan is mutagenic (a clastogenic agent, which induces DNA damage) and another potential cause for cancer in this study group. Although Busulfan is frequently used in combination with cyclophosphamide in the clinical setting, there is the risk that it independently can cause cancer, even with the low dose proposed in this study. Also, there is the possibility that a subject will be exposed to busulfan without receiving the gene-modified cells.

Insertional oncogenesis (IO):

Risks from IO have occurred in clinical trials of gene transfer using γ-retroviral vectors. After immune reconstitution in 9 of 10 subjects participating in an X-SCID gene transfer study in Paris, France (Cavazzana-Calvo M et al 2000; Hacein-Bey-Abina S et al 2002), four of these subjects developed leukemia ≥ 2½ years after receiving the gene-transduced CD34+ cells (Hacein-Bey-Abina, S et al., 2003A; Hacein-Bey-Abina, S et al., 2003B; Hacein-Bey-Abina S et al., 2008). In a similar trial of gene therapy for X-SCID performed in London, England, one of 10 subjects also developed T lymphoproliferation (Howe, 2008). One of these five patients who developed T lymphoproliferative disease (LPD) has died and the other four are in leukemia remission and continue to have restored immunity from the gene therapy. For three of the subjects with leukemia, the proviral insertion sites have been mapped within or near (3 kb) of the LMO-2 gene (Hacein-Bey-Abina, S., et al, 2003), a human proto-oncogene associated with T-cell leukemia (Neale et al. 1997; Mead et al. 2001; Rabbitts, T. H. et al. 1998). In a fourth case, two integrations

sites were described: LMO-2 and BMI1, which is also a proto-oncogene. In a fifth subject, vector had integrated near a third proto-oncogene, CCND2 (Hacein-Bey-Abina S et al., 2008). These are the first reported cases of leukemia developing in subjects participating in a clinical study of γ-retroviral-mediated gene transfer to CD34+ cells. Additionally, a monkey infused with hematopoietic progenitors exposed to a γ-retroviral vector died from granulocytic leukemia, probably due to vector insertional oncogenesis (Seggeweis et al, 2006). The probability of leukemia developing in other participants of the French trial, or in participants of other trials, is not known. To date (10/08/12), 17 ADA-deficient SCID patients have undergone these protocols in Milan and six in London (by personal communication from site PIs) and 14 in the CHLA/NIH and UCLA/NIH trials. No cases of T LPD have occurred in the ADA subjects, in sharp contrast to the X-SCID results.

Currently, there have been no reported cases of insertional mutagenesis in clinical trials using a lentiviral vector for gene therapy. Themis et al (2005) observed oncogenesis using lentiviral vector-mediated gene therapy in fetal and neonatal mice, but this has not been shown in humans nor primate models. Modlich (2009) showed that the risks for insertional mutagenesis are lower with lentiviral vectors that lack strong γ-retroviral LTR *(as in the EFS-ADA vector) compared to γ-retroviral vectors with intact LTR.

Bone marrow harvest:

The principal risk of the bone marrow removal is associated with having general anesthesia. General anesthesia can cause problems, such as allergic reactions, breathing or heart problems and, rarely, the possibility of death. Other risks include bleeding, pain, or infection at the bone marrow removal sites. Subjects will receive pain medication to relieve pain as necessary. Antibiotics will be administered as soon as possible if an infection is detected. Blood tests will be done 1-3 days prior to the bone marrow harvest to be sure that subjects do not have a greater risk for bleeding. There is a possibility that the bone marrow harvest will make subjects anemic enough to require a blood transfusion.

CD34+ cell infusion:

At the time the transduced cells are infused back into the bloodstream, the subjects may experience fever, chills, bronchospasm, and rarely, an anaphylactic reaction, which could be fatal.

Replication-competent lentivirus (RCL) exposure or other microbial contaminants:

The specific preparations of lentiviral vector supernatant used to transduce the CD34+ cells will have been screened to ensure the absence of replication-competent lentivirus (RCL). Replication-defective lentiviral vectors do not cause any known disease in human beings. However, there is a small possibility and risk from this treatment that undetected RCL are present, which could cause an active infection in subjects. However, the residual HIV-1 sequences without any open reading frames remaining in the lentiviral vector backbone would not be expected to be able to cause pathology.

It is also possible that the cells could become infected with bacteria or fungus during the growth period in the laboratory. If there is any evidence of infection of the cells with unwanted agents and Busulfan has already been administered, the cells will not be returned to the subject, and the

back-up bone marrow will be infused. However, there is a remote chance that subjects could acquire a bacterial or fungal infection from the re-infused cells (this also applies to the back-up cells, if administered).

Allergic/immunological responses to cell processing excipients:

Stem Cell Factor (SCF) and Megakaryocyte Growth and Development Factor (MGDF), also called thrombopoietin (TPO) are used to maintain stem cell viability and to promote gene transfer during the *ex vivo* transduction process. When these factors are administered parenterally, they may have untoward immunologic or allergic consequences such as thrombocytopenia or allergic reactions. The CD34+ cells cultured in these cytokines are thoroughly washed following *ex vivo* exposure. Since small amounts of residual factors may remain, it is possible that the presence of these may result in an adverse immunologic or allergic event. This hypothetical complication has not been reported from any of the clinical trials using these factors for *ex vivo* transduction.

Germ-line transmission of vector sequences:

Issues of contraception and pregnancy are moot in infants and children before puberty. Since the effects of the lentiviral vector on the fetus is unknown, females of child-bearing potential are to use an effective means of birth control during the treatment phase of the study and for at least six months after the cell infusion. Similarly, males are to use at least a barrier method of contraception. Females of child-bearing potential must have had a negative pregnancy test within three days prior to bone marrow harvest, the morning before receiving Busulfan and again the morning of and prior to, cell reinfusion. Because we will only be putting the EFS-ADA vector into the CD34+ cells from bone marrow *ex vivo*, we do not anticipate that they can enter the germ cells.

Other adverse clinical consequences:

Participation in this protocol may have deleterious effects on subsequent attempts at allogeneic HSC transplantation. These adverse effects could be due to "immunization" to normal human ADA produced from the transgene. Most patients, however, will have received bovine PEG-ADA, which is more likely to be immunogenic than human ADA; the development of significant inhibitory antibodies has been relatively rare in patients treated with PEG-ADA alone. It is possible that non-PEGylated human ADA could be more immunogenic or its expression in cells could induce more T cell responses than injected recombinant protein. Additionally, this protocol could lead to restoration of partial immunity, insufficient for complete protection from infections and yet sufficient to increase the risks of rejections of allogeneic cells. This partial immunity may also result from PEG-ADA alone, although it may be possible to eradicate this residual activity by complete PEG-ADA cessation. For patients who have not received PEG-ADA the gene transfer procedure would introduce these risks de novo, although it is difficult to know *a priori* the potential likelihood of these hypothetical risks.

As in any new form of therapy, there may be risks which are unknown or not anticipated. More importantly, the prior 16 patients that were treated by a similar protocol by these investigators did not experience any permanently debilitating nor deleterious side effects.

2.3.2 Potential Benefits

At this stage, the procedure is experimental. However, similar studies using γ-retroviral vectors to transfer an ADA cDNA into bone marrow CD34+ cells done in performed in Italy, the UK and by our group in the US resulted in clinical benefits in the majority of subjects. It is too early to determine whether these patients will have durable, life-long ADA gene expression/enzyme production and sustained immune function. If the gene transfer using the EFS-ADA lentiviral vector is more effective than with the γ-retroviral vectors, then it is possible that the subjects will develop improved immune function and not require either a bone marrow transplant or further PEG-ADA therapy.

3 STUDY OBJECTIVES

3.1 Primary Objectives

The primary objectives are;

- to examine the safety of autologous transplantation of bone marrow CD34+ cells transduced with the EFS-ADA lentiviral vector (OTL-101). The safety review will include the identification of grade III/IV serious adverse events (SAE). Subjects will also be monitored for clinical toxicities, replication competent lentivirus, and monoclonal expansion at regular intervals by a clinician, as well as standard blood counts and chemistries.
- to estimate overall and event-free survival by 12 months where failure is defined by one of the following endpoints: death; reinstitution of PEG-ADA; or performance of an allogeneic BMT.

3.2 Secondary Objectives

The secondary objectives will assess the efficacy of OTL-101, engraftment of transduced cells and the extent of ADA expression and immune reconstitution. The secondary outcomes will:

1. Assess overall and event free survival at 24 months,
2. Compare overall survival and event free survival at 24 months between patients treated with OTL-101 and patients treated with allogeneic HSCT,
3. Assess the extent of gene transfer in peripheral blood cells,
4. Assess ADA gene expression by measuring ADA enzymatic activity and adenine nucleotides,
5. Examine the effects of reconstituting ADA gene expression on immune function through serial examination of peripheral blood leukocytes and myeloid cells,
6. Assess immune reconstitution and infection rates, and
7. Assess use of immunoglobulin replacement therapy.

4 STUDY DESIGN

4.1 Description of the Study Design

This is a controlled, non-randomized Phase I/II clinical trial to assess the safety and efficacy of autologous transplantation of CD34+ cells from the bone marrow of ADA-deficient SCID infants and children following human ADA cDNA transfer by the EFS-ADA lentiviral vector.

Following provision of informed consent, enrolled subjects will be screened to determine full eligibility for participation over 1-2 weeks. Eligible subjects will undergo bone marrow harvest under general anesthesia. The marrow will be processed to isolate CD34+ cells and transduce them with the EFS-ADA lentiviral vector. If sufficient cells are obtained, the subjects will undergo marrow cytoreduction with busulfan (4 mg/kg). If OTL-101 meets all release criteria, the cells will be infused IV. PEG-ADA ERT will be discontinued at day +30. The initial portion of the study will require the subject to be treated in the hospital for a typical duration of 10 days-6 weeks. After discharge from the hospital, the subject will be seen for interval history and examination by either their home physician, the PI or a CI and have blood drawn at months 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 21, and 24. Subjects will then be consented for a long-term follow-up study that consists of semi-annual to annual visits and lab draws over the subsequent 13 years.

The initial purpose of this study is to determine the safety of the procedure in which CD34+ cells from bone marrow of ADA-deficient SCID patients are transduced by lentiviral-mediated transfer of a normal human ADA cDNA, infused intravenously, and engrafted to produce mature peripheral blood leukocytes containing and expressing the ADA cDNA. Secondary objectives will assess the efficacy of this procedure. Potentially, the development of lymphocytes expressing ADA could restore functional immunity in the absence of exogenous PEG-ADA enzyme replacement therapy.

4.2 Study Endpoints

4.2.1 Primary Endpoints

4.2.1.1 Evaluation of Safety

Primary Endpoint: Incidence and grade of Serious Adverse Events (SAE).
Serious adverse events may be manifest as:

a) clinical toxicities

b) exposure to replication-competent lentivirus (RCL)

c) development of monoclonal expansion or leukoproliferative complications from vector insertional effects.

a) To detect potential clinical toxicities from the treatment, patients will have interval medical histories taken, complete physical examinations, and phlebotomy to obtain peripheral blood for laboratory studies on day +1, and then at months 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 21 and 24 (+/- 4 weeks). Laboratory studies performed at these time-points will include CBC with differential and platelet count and a chemistry panel and electrolytes. Toxicities relative to the clinical parameters, the CBC with differential, and the chemistry panel are defined by an adapted Pediatric Clinical Toxicity Scale from the NIAID, Division of AIDS. **(Appendix G)**

Patients will also be monitored by clinical examination and laboratory studies in the immediate period (3 to up to 6 weeks, depending on clinical course) after infusion of OTL-101 to monitor myelosuppression expected from the administered busulfan.

b) To detect exposure to replication-competent lentivirus:

i. PBMC will be assayed by PCR to detect RCL. This test will be performed by the National Gene Vector Biorepository on samples obtained at baseline (pre-infusion), 3, 6, 12 and 24 months post-infusion. Samples will also be collected annually thereafter, and cells archived for PCR assay, to be run if any samples are positive.

ii. Serum for measurement of antibody to RCL will be archived for the presence of antibody to VSV-G envelope at baseline (pre-infusion), 3, 6, 12 and 24 months post-infusion (and annually thereafter).

c) To monitor for monoclonal expansion or leukoproliferative complications, LAM-PCR will be performed, as described in Appendix F.

4.2.1.2 Evaluation of Efficacy

Primary Efficacy: Overall/event-free survival (survival without need for BMT or PEG-ADA).

Overall/event-free survival will be determined for each subject by 12 months where failure is defined by one of the following endpoints: death; reinstitution of PEG-ADA; or performance of an allogeneic BMT.

4.2.2 Secondary Endpoints

The secondary endpoint is overall survival and event free survival at 24 month.

The other Secondary efficacy falls under the following outcomes or categories:

1. Efficacy of gene transfer/engraftment of HSC
2. ADA expression, enzyme activity and detoxification
3. Effects on ADA reconstitution on immune phenotype and function (the need for IgRT will be assessed under this category)
4. Immune reconstitution
5. Infection rates

1. Efficacy of gene transfer/engraftment of HSC

Secondary Efficacy Endpoint 1: Determine the frequency of gene marking in PB cells.

To assess the efficacy of stem cell transduction/engraftment by serial examination of peripheral blood lymphocytes and myeloid cells to quantify the percentages of cells containing the ADA cDNA by quantitative DNA-PCR. We will obtain serial samples of peripheral blood and fractionate the leukocytes on ficoll-hypaque to obtain peripheral blood mononuclear cells (PBMC) and granulocytes. Genomic DNA isolated from each cell population will be assayed for the frequency of cells containing the EFS-ADA vector by quantitative PCR (qPCR). If sufficient cells are available, then the PBMC cells may be sub-fractionated by immunoaffinity or FACS sorting into T

cells (CD3+), B cells (CD19+), NK cells (CD16+/CD56+) and myeloid cells (CD13+/CD14+, or CD33+) and DNA from these cell sub-populations assayed by qPCR for vector marking.

The presence of transduced peripheral blood leukocytes will demonstrate the transduction and engraftment of the manipulated CD34+ cells. Absence of transduced cells will indicate failure of transduction and/or engraftment. Because of the rapid turnover of mature myeloid cells, the continued presence (>6 months) of transduced granulocytes and monocytes, will be taken as evidence of transduced/engrafted long-lived stem cells. Increased levels of transduced T and B lymphocytes, above the level of transduced myeloid cells, will indicate a selective advantage to T and B progenitors expressing the normal ADA gene.

Secondary Efficacy Endpoint 2: Quantitate clonal diversity of vector integrants.

The clonal diversity will be quantitated and used as an index of the numbers of vector transduced HSC that engrafted in the subjects. The number of retroviral vector (for the prior and current trials) and lentiviral vector (for the proposed trial) integration sites will be amplified by a modified nrLAM-PCR protocol (Paruzynski, et al, 2010) and sequenced via Illumina GAIIx high-throughput sequencers. Custom software code has been written to process raw sequence reads and determine the genomic positions of integration sites. We expect to identify >1x 10^5 integration sites per experiment, which will lend significant statistical power to downstream analysis.

2. ADA expression and enzyme activity

Secondary Efficacy Endpoint 3: ADA enzyme activity in erythrocytes.

ADA enzymatic activity in erythrocytes will be measured by Michael Hershfield's CLIA-certified laboratory at Duke University, Durham, North Carolina as a clinical test. Attainment (yes/no) of levels compared to the normal reference range (63±41 nmol/h/mg) will be evaluated.

Secondary Efficacy Endpoint 4: Total adenine nucleotides in erythrocytes.

The levels erythrocyte deoxyadenosine nucleotides will be determined at each time point (at the laboratory of Dr. Michael Hershfield, Duke University). The level of deoxyadenine nucleotides in erythrocytes provides an indirect assessment of systemic detoxification by ADA enzyme activity. While not a primary study end-point, these parameters will be measured to provide additional information on the effects of the gene transfer.

3. Effects on ADA reconstitution on immune phenotype and function

These immunological assays will provide a laboratory assessment of immune function after the gene transfer procedure.

Secondary Efficacy Endpoint 5: Absolute lymphocytes on CBC

Absolute lymphocyte counts (ALC) will be determined by routine clinical laboratory complete blood counts (CBC).

Secondary Efficacy Endpoint 6: Absolute number T, B, and NK lymphocytes

Absolute number T, B, NK lymphocytes in peripheral blood will be determined by flow cytometry.

Secondary Efficacy Endpoint 7: Lymphocyte mitogenic proliferation

T lymphocyte proliferative responses to mitogen (phytohemagglutinin – PHA) and to antigens (tetanus toxoid after vaccination and Candida albicans) will be determined by tritiated thymidine incorporation or carboxyfluorescein succinimidyl ester (CFSE) labeling by routine clinical laboratory assay.

Secondary Efficacy Endpoint 8: Quantitative immunoglobulins by class

Serum immunoglobulin levels (IgG, IgA and IgM) will be determined by routine clinical laboratory assays. Requirement of immune replacement therapy will be assessed

Secondary Efficacy Endpoint 9: Specific Antibody responses

Serum titers of isohemagglutinin antibodies and antibodies to vaccine antigen tetanus toxoid and to polyribosylphosphate {PRP} will be determined by routine clinical laboratory tests if vaccinations have been administered.

Secondary Efficacy End-point 10: T lymphocyte reconstitution

T lymphocyte production and diversity will be analyzed by measuring PBMC TREC by qPCR and vector integration sites by LAM-PCR, and TCR Vβ family usage by FACS.

Secondary Efficacy End-point 11: Infection rates will be also measured under immune reconstitution as part of the efficacy assessment of a competent immune system

5 STUDY POPULATION

5.1 Description of the Study Population

Number of subjects to be studied:

A total of twenty (20) subjects will be treated under this protocol. Eligible subjects will be children ≥ 1.0 months of age with a diagnosis of ADA-deficient SCID, lacking a medically eligible HLA-matched sibling donor and meeting defined inclusion/exclusion criteria related to medical status and organ function (*vide infra*).

5.1.1 Participant Inclusion Criteria

Inclusion criteria:

Participants must satisfy Inclusion Criteria I, II, and III.

I. Children ≥ 1.0 months of age with a diagnosis of ADA-deficient SCID based on:

A. Decreased ADA enzymatic activity in erythrocytes, leukocytes, skin fibroblasts, or in cultured fetal cells to levels consistent with ADA-deficient SCID as determined by reference laboratory **or** confirmed ADA gene mutation(s) known to cause disease

AND

B. Evidence of severe combined immunodeficiency based on either:

1. Family history of first order relative with ADA deficiency and clinical and laboratory evidence of severe immunologic deficiency,

OR

2. Evidence of severe immunologic deficiency in subject prior to institution of immune restorative therapy, based on

    a. lymphopenia (absolute lymphocyte count <400 cells/mcL) OR absence or low number of T cells (absolute CD3+ count <300 cells/mcL)

    OR

    b. severely decreased T lymphocyte blastogenic responses to phytohemagglutinin (either <10% of lower limit of normal controls for the diagnostic laboratory, <10% of the response of the normal control of the day, or stimulation index <10)

II. Ineligible for matched sibling allogeneic bone marrow transplantation:
Absence of a medically eligible HLA-identical sibling, with normal immune function, who may serve as an allogeneic bone marrow donor.

III. Signed written informed consent according to guidelines of the IRB (UCLA Office of Human Research Protection Program and National Human Genome Research Institute (NHGRI) Institutional Review Board

5.1.2 Participant Exclusion Criteria

Exclusion criteria:

1. Age ≤ 1.0 months

Appropriate organ function as outlined below must be observed within 60 days of entering this trial.

2. Hematologic

a. Anemia (hemoglobin < 10.5 g/dl at < 2 years of age, or < 11.5 g/dl at > 2 years of age).

b. Neutropenia: absolute granulocyte count <500/mm^3.

c. Thrombocytopenia (platelet count < 150,000/mm^3, at any age).

d. INR or PT > 2X the upper limit of normal or PTT > 2.33X the upper limits of normal (patients with a correctable deficiency controlled on medication will not be excluded).

e. Cytogenetic abnormalities on peripheral blood or bone marrow or amniotic fluid (if available).

f. Prior allogeneic HSCT with cytoreductive conditioning.

3. Infectious

a. Evidence of infection with HIV-1, hepatitis B, Hepatitis C, or parvovirus B 19 by DNA PCR within 90 days prior to bone marrow harvest. If other infection is present, it must be under control (e.g. stable or decreasing viral load) at the time of screening.

4. Pulmonary

a. Resting O_2 saturation by pulse oximetry < 95% on room air.

b. Chest x-ray indicating active or progressive pulmonary disease.

5. Cardiac

a. Abnormal electrocardiogram (EKG) indicating cardiac pathology.

b. Uncorrected congenital cardiac malformation with clinical symptomatology.

c. Active cardiac disease, including clinical evidence of congestive heart failure, cyanosis, hypotension.

d. Poor cardiac function as evidenced by LV ejection fraction < 40% on echocardiogram.

6. Neurologic

a. Significant neurologic abnormality by examination.

b. Uncontrolled seizure disorder.

7. Renal

a. Renal insufficiency: serum creatinine $\geq$ 1.2 mg/dl, or $\geq$ 3+ proteinuria.

b. Abnormal serum sodium, potassium, calcium, magnesium, phosphate at grade III or IV by Division of AIDS Toxicity Scale.

8. Hepatic/GI:

a. Serum transaminases > 5X the upper limit of normal (ULN).

b. Serum bilirubin > 2X ULN.

c. Serum glucose > 1.5x ULN.

d. Intractable severe diarrhea.

9. Oncologic* (see below)

a. Evidence of active malignant disease other than dermatofibrosarcoma protuberans (DFSP)

b. Evidence of DFSP expected to require anti-neoplastic therapy within the 5 years following the infusion of genetically corrected cells

c. Evidence of DFSP expected to be life limiting within the 5 years following the infusion of genetically corrected cells

10. Known sensitivity to Busulfan

11. General

a. Expected survival < 6 months.

b. Pregnant.

c. Major congenital anomaly.

d. Ineligible for autologous HSCT by the criteria at the clinical site.

e. Other conditions which in the opinion of the principal investigator and/or co-investigators, contra-indicate the bone marrow harvest, the administration of busulfan, infusion of OTL-101 or indicate the patient or patient's parents/primary caregivers inability to follow protocol.

* DFSP is a rare, locally invasive tumor with low metastatic potential. Patients receiving active anti-neoplastic therapy for any cancer, including DFSP, are not eligible. Patients with DFSP who are not being treated with active anti-neoplastic therapy at the time of enrollment AND have no plan to receive active anti-neoplastic therapy in the absence of progressive malignant disease AND whose DFSP is not expected to be life-limiting within the five years following the infusion of genetically corrected cells are eligible.

Patients with DFSP, for whom radiation or chemotherapy has been chosen, would remain ineligible during treatment, as the interaction of busulfan and the experimental gene transfer vectors with active anti-neoplastic therapy is difficult to predict and could reasonably be expected to be deleterious due to overlapping toxicities. When anti-neoplastic therapy is concluded, patients with ADA-SCID and a history of DFSP can be included.

5.1.2.1 Co-enrollment Guidelines

Co-enrollment:

We will request that subjects do not enroll in any other gene therapy for 2 years after the autologous infusion. Since this is a Phase I/II clinical trial, we do not have enough information to predict any interactions with other study agents. We will allow enrollment in other data collection, sample banking, and registry studies.

5.2 Strategies for Recruitment and Retention

Recruitment:

We will promote recruitment by posting the study on the NIH website for clinical trials, http://www.clinicaltrials.gov. We will also make our best efforts to present our trial at national meetings for Immunology, Gene and Stem Cell Therapies, and Bone Marrow Transplantation.

Retention:

This patient population will require close clinical monitoring after the initial admission for the harvest, transduction, and re-infusion of cells to observe their immune function status. To attempt to retain subjects for follow-up evaluations, after completing the informed consent process, we will also have multiple discussions with the patient's parent(s)/legal representative (the patients are usually minors) and primary physician so that they have a good grasp of the treatment plan and what it entails and the rationale for the proposed follow-up studies.

6 STUDY AGENT/INTERVENTIONS

The Investigational New Drug to be tested is autologous CD34+ cells transduced *ex vivo* with a lentiviral vector based upon the self-inactivating (SIN) lentivirus (HIV-based LV): EFS-ADA

(referred in this document as OTL-101). The vector has been produced as cell-free lentiviral supernatant from the 293T cell line, and has an envelope pseudotype from the vesicular stomatitis virus (VSV). The vector will be used for *ex vivo* transduction of CD34+ progenitor cells from the bone marrow of infants or children with adenosine deaminase (ADA)-deficient severe combined immunodeficiency (SCID). OTL-101 will be subsequently infused intravenously after a single non-myeloablative dosage of busulfan (4 mg/kg intravenously x1).

This is a Phase I/II Clinical Trial to evaluate the safety (as primary endpoint) and efficacy (as secondary end-points) of the addition of a normal human ADA cDNA by the EFS-ADA lentiviral vector to bone marrow CD34+ stem/progenitor cells. Due to the natural course of the disease, these patients require some form of treatment and with that in mind, a placebo group would be unethical. At the current time, the standard of care is a matched sibling allogeneic bone marrow transplantation, when a medically-suitable donor exists. Since our agent is still under investigation, if a patient has an HLA matched sibling and qualifies for a bone marrow transplantation, they will be excluded from our study. The other current treatment options have their drawbacks and limitations, including high financial cost, need for regular IM injections (PEG-ADA) or GVHD, dysfunctional immune system, and death from unrelated or mismatched HSCT (described in more details above).

An “Appendix M” has been submitted to the Recombinant DNA Advisory Committee (RAC) as per the Office of Biological Activities (OBA), RAC guidelines and unanimous RAC recommendation for approval was obtained on Dec 2, 2009 (#0910-1006).

6.1 Study Agent Acquisition

The study agent is autologous bone marrow CD34+ cells transduced with the EFS-ADA lentiviral vector (OTL-101). The CD34+ cells will be isolated from the bone marrow of each subject. The EFS-ADA lentiviral vector preparation will be produced at the Indiana University Vector Production Facility (IUVPF), Indianapolis IN. The vector supernatant will be shipped to UCLA and the NIH on dry ice under UN 2814 (infectious substances) and UN 1845 (dry ice).

Cytokines used for ex vivo stimulation of the CD34+ cells during transduction will be purchased from commercial sources and qualified for use following guidelines of the FDA. Recombinant fibronectin fragment CH-296 will be supplied from Takara Shuzo, Co, Otsu Japan. CD34+ cells will be isolated using the CliniMacs from Miltenyi (with cross-reference to their IDE).

A. CD34+ Cell Isolation and Transduction with EFS-ADA

CD34+ Cell Isolation

The bone marrow will be collected from the subjects in an operating room in the Ronald Reagan Medical Center (RRMC). The bone marrow will be transported from the surgical collection site to the Bone Marrow/Hematopoietic Progenitor Stem Cell (BM/HPSC) Laboratory (46-126 CHS), according to RRMC SOP, for initial processing (sterility sampling and cell count). The back-up bone marrow (5×10^7 TNC/kg, or 3×10^7 ficolled MNC/kg) will be removed, if necessary*, and cryopreserved according to the SOP of the BM/HPSC Lab. The bone marrow will be transported

to the GMP facility (14-246 Factor), according to the Clinical Gene Therapy Laboratory SOP (#7000), for CD34+ cell isolation and transduction.

At NIH, all steps for the bone marrow processing for CD34+ cell isolation and EFS-ADA lentiviral vector transduction are performed at the Cell Processing Section (CPS) of the Department of Transfusion Medicine in the NIH Clinical Center.

Bone marrow mononuclear cells will be separated using the standard ficoll-hypaque centrifugation method. The buffy coat layer will be collected and processed to isolate CD34+ cells, using the Miltenyi CliniMACS®, following the manufacturer's instructions. The collected CD34+ cell fraction will be characterized for total cell number, % of CD34+ cells, and CFU-GM content by CFU assay in methylcellulose. If the resulting cell number is $>5x10^5$ and $<1x10^6$ CD34+ cells/ kg, the isolated CD34+ cells will be cryopreserved as additional back-up, and the subject will be offered the option for a second marrow harvest at least one month later. If sufficient CD34+ cells are obtained from the second harvest ($\geq 1x10^6$/kg), the procedure may proceed. If insufficient cells are obtained from both harvest(s), the subject will be withdrawn from further study participation. If sufficient CD34+ cells are isolated ($\geq 1x10^6$ / kg), the cells will be processed for gene transduction.

* If the subject's umbilical cord blood (UCB) was cryopreserved by a clinical stem cell lab at the time of birth, the back-up cells may be allocated from the UCB. If no UCB was collected or if less than $5x10^7$/kg TNC are contained in the UCB at the time of cryopreservation, all (if no UCB) or the remainder (if insufficient TNC from the UCB) of the back-up cells will be obtained from the bone marrow at the time of harvest.

B. Certificate of Analysis for Final cell Product

CHARACTERIZATION OF OTL-101

Assays during transduction:	Results
In-process daily bacterial culture	no growth*
In-process daily fungal culture	no growth*
Assay of culture medium at completion of transduction:	
Assay for RCL (archived and done if necessary)	no RCL detected
Assays of cells at completion of transduction:	
Cell number[1]	≥1x10e6 nucleated cells/kg*
Viability	≥70% trypan blue exclusion*
Gram stain of OTL-101	no bacteria seen*
Endotoxin assay of final cell product	less than 5 EU/kg/hr*
Bacterial culture of final cell product	no growth, Δ 14 days
Fungus culture of final cell product	no growth, Δ14 days
Mycoplasma PCR[¥]	not detected
Assay for RCL by PCR on cell pellet DNA & co-cultivation on 1% final cell product (archived & done if necessary) [2]	no RCL detected
FACS analysis of %CD34+	to be determined
Vector copy number[3]	to be determined
Identity assay by PCR	presence of expected band

* Release criteria. Other values will be known after cell infusion.
[¥]Performed by Labs, Inc (Centennial, CO) or Rapid mycoplasma detection method of BREL Method Number 300200GMP.BSV
[1]Cell counts and viability done by trypan blue/hemacytometer or automated cell counter in clinical lab (UCLA).
[2] Cells and medium will be archived for assay of RCL; if any PBMC samples are positive by qPCR for VSV-G sequences, then RCL assays would be run on these archived samples
[3] Performed by ddPCR or qPCR on post-transduction bulk cells after 2 weeks culture

For female subjects of child-bearing age, a serum pregnancy test performed the morning prior to infusion must be negative.

C. Action plan for positive culture result from transduction

The final cell product is tested by doing gram stain, endotoxin testing for bacteria, and cultures for bacteria and fungi and mycoplasma PCR. If the gram stain or endotoxin is positive, then the cells will not be infused and the stored back-up will be infused. Should any of the culture results return positive after the product has been infused, the PI would be immediately notified. The subject will most likely still be hospitalized and will be checked for constitutional symptoms (fever, chills, malaise, fatigue). If any signs or symptoms of bacterial

infection exist, the subject would have complete cultures of blood, urine, sputum done and chest X-ray performed, if clinically indicated, and broad-spectrum antibiotics initiated. Should any cultures return positive, antimicrobial therapy would be tailored to the sensitivity of the specific organism. If cultures of the final product or of components used in the gene transfer matched the culture results of the subject's samples, the FDA and the IRB would be notified for further action plans, and the trial put on hold until the source of contamination is confirmed.

D. Dosage Calculation

After subtraction of the back-up (5×10^7 whole BM TNC / kg, or 3×10^7 ficolled MNC/kg), the criteria for sufficient cell number to proceed with Busulfan conditioning is:

The successful isolation of CD34+ cells from treatment phase bone marrow harvest is defined as ≥ 1×10^6 /kg. If between $5x10^5$ and $1x10^6$ cells are obtained, they will be cryopreserved as additional back-up and the subject offered a second marrow harvest. If the CD34+ cell yield from the second harvest is ≥ $1x10^6$/kg, the cells will be transduced with the EFS-ADA lentiviral vector. If < 1×10^6 /kg cells are obtained from the second harvest, the subject will be withdrawn from further participation in the study.

This number is derived as follows:

A standard estimate of the minimal numbers of allogeneic bone marrow total nucleated cells used for transplant is at least 5×10^7 cells/kg of recipient weight. This cell number (or 3×10^7 ficolled MNC/kg) will be frozen as a back-up. The back-up will be used if OTL-101 does not meet infusion specifications, or if there is engraftment failure evident after +42 days post cell infusion [ANC < 200 or platelet count < 20,000, repeated and confirmed x3 total determinations). With CD34+ cells comprising approximately 1.0% of the mononuclear cells in marrow, this translates to an average number of back-up CD34+ cells being approximately 0.5×10^6/kg. CD34+ cells will be isolated from bone marrow TNC in excess of 5×10^7/kg or ficolled MNC in excess of 3×10^7/kg. [NOTE: If the subject's umbilical cord blood (UCB) was cryopreserved by a clinical stem cell lab at the time of birth, the backup cells may be allocated from the cord blood. If less than $5x10^7$/kg TNC are contained in the cord blood at the time of cryopreservation, the remainder of the backup cells will be obtained from the bone marrow at the time of harvest. If $5x10^7$/kg TNC are obtained from the UCB for the backup, the volume obtained from the bone marrow harvest at the time of treatment, less 2 ml required for characterization, will be used to isolate CD34+ cells for the transduction procedure.] The cells will then be transduced with the vector and, after washing, given back to the subject pending meeting the release criteria. If cells do not meet release criteria, they will not be infused. If the subject has already received Busulfan, the subject will be administered only the non-transduced back-up cells.

Therefore, these dosage calculations will be used for assessment of our ability to achieve the end-points. It is difficult to specify the minimal number of transduced CD34+ cells to be infused, because it is possible that successful engraftment of transduced stem cells may be achieved with lower numbers of engrafting, transduced CD34+ cells than those set by arbitrary criteria. We consider the minimal acceptance criteria for infused, transduced cells to be 0.5×10^6 total cells / kg.

It should be noted that since the subjects will receive prior non-myeloablative conditioning, we will cryopreserve non-transduced cells. These will be infused if the final product contains **<** 0.5 x 10^6 cells / kg. If necessary, these back-up bone marrow cells would be re-infused to overcome more severe myelosuppressive effects of the conditioning regimen (persistent ANC < 200/µl or platelets < 20,000/µl after day +42 from the day of cell infusion on three independent and consecutive determinations over at least ten days). It is recognized that there is no specific information available to extrapolate from the traditional, but empirically-based, numbers of cells needed to overcome allogeneic transplant barriers to the number of *ex vivo*-transduced, autologous cells needed for engraftment and therapeutic effects. Similarly, it should be recognized that transduction of clonogenic progenitors poorly predicts transduction of pluripotent stem cells, which can only be assessed by transplantation into recipients and observation of cells produced over subsequent months and years.

Therefore, these observations of the numbers of CD34+ cells obtained and the percentages that become transduced will be recorded. However, if < 1 x 10^6 CD34+ cells/kg are isolated after two harvests, subjects will be withdrawn from the study and not receive busulfan. We will limit total infusion dose to 15 x 10^6 cells per kg, as approved by the FDA/CBER. If more than 15 X 10^6 cells per kg are isolated after the culturing period, the gene-modified cells that remain after the total maximum dose has been given will be disposed or stored for research.

6.1.1 Study Agent/Intervention

6.1.1.1 Formulation, Packaging, and Labeling

The study agent is manufactured for individual-specific use with the individual's (subject's) autologous cells. Manufacturing will take place in the GMP facility of the Human Gene Medicine Program (HGMP) at UCLA or at the Cell Processing Section of the Department of Transfusion Medicine (CPS, DTM) of the NIH Clinical Center (FDA registration number 1174694). Both sites will use the same transduction protocol and the same lots of reagents (e.g. vector supernatant, cytokines, retronectin). With the exception of vector DNA PCR and ADA enzyme activity, which will be the same at both sites, tests for the Certificate of Analysis (e.g. cell counts, viability, sterility, endotoxin, CD34 enumeration, and CFU analysis) will be according to each sites' SOPs. As UCLA is the lead site, the NIH site will be qualified by the Protocol Manager on behalf of the Sponsor to ensure that operations at the NIH are comparable. Additionally, the NIH will perform validation runs to ensure that the endpoints of the Final Certificate of Analysis can be met.

Following the transduction procedure, the cells are washed and resuspended in Plasma-lyte A with 1% human serum albumin (HSA). This "final product" is loaded into a sterile syringe, capped, and labeled using the following example label or similar:

EFS-ADA TRANSDUCED CELLS (IND 15440)

CAUTION: New Drug – Limited by Federal Law for Investigational Use

PIN: _____ Medical Record #: _____

Date: _____ Time: _____am / pm

Product Type: Genetically modified autologous BM CD34+ cells in Plasma-Lyte A+1%HSA vol. _____ml

total nucleated cells: _____Product Nr: _____

FOR AUTOLOGOUS USE ONLY. NOT EVALUATED FOR INFECTIOUS SUBSTANCES. Properly identify intended recipient and component or unit.

Checked by:_____

WARNING: This product may transmit infectious agents.
DO NOT IRRADIATE

University of California, LOS ANGELES
LOS ANGELES, CA 90095


Biohazard

The study agent is stable indefinitely at room temperature and is administered within 3 hours of its preparation. The entire dose of cells that is manufactured for each subject (minus ~10% for quality control) will be administered to the subject by I.V. injection.

6.1.1.2 Preparation, Administration, Storage, and Dosage of Study Agent(s)/Intervention(s)

The study agent is prepared on the day of administration. Following the transduction procedure, in which the CD34+ cells have been in culture for approximately 36-48 hours, the cells are harvested, washed with Hank's Balanced Salt Solution (HBSS) + 1% HSA and resuspended in 25 mL of Plasma-lyte A + 1% HSA and placed in a sterile syringe. This "final product" is kept at room temperature and transported to the infusion site (usually hospital room) in a small "Igloo"-type cooler, or equivalent per site SOP. The study agent will be administered within 3 hours of its preparation via I.V. injection over 5-15 minutes. During the administration period and afterwards, the subject will be monitored for any adverse events, such as allergic reaction(s). Tylenol™ and Benadryl™ will be administered approximately ½ hour prior to administration of the study agent.

The entire contents of the syringe will be administered; there will be no need to store and re-use "left-over" cells. The dose of OTL-101 that will be given is a one-time administration of 1 X 10^6 to 15 X 10^6 total cells/kg. If the number of cells harvested after culturing exceeds 15 X 10^6 cells/kg, the remaining cells with be frozen and used for research or discarded, but they will not be thawed at a later date to give to the subject. Because the study agent is a biologic, care must be taken in handling this product, such as using gloves. After the cells have been administered, the empty syringe will be disposed of as biohazardous waste.

6.1.1.3 Study Agent Accountability Procedures

The study agent is manufactured on an individual basis for subject-specific use. All of the manufactured product (less ~10% for QC) will be administered to the subject one time only. A clinical investigator or registered nurse will administer the study agent by I.V. infusion. Thus, the study agent will not be distributed to subjects for self-administration.

The reagents used to produce the final cell product (EFS-ADA lentiviral vector, recombinant cytokines, recombinant fibronectin) will be kept under locked restricted access only to the study investigators.

6.2 Assessment of Participant Compliance with Study Agent(s)/Intervention(s)

The only time the subjects will be exposed to the study agent is the day they receive their gene-modified cells. The subjects are required to make follow-up visits, either to us or to their personal physician, at day 1 and months 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 21 and 24 post-infusion of gene-modified cells for a physical exam and blood draw only (i.e. no more administration of study agent). These follow-up visits are scheduled by the physician's office by actively contacting the subject/subject's parents/legal representative.

6.3 Concomitant Medications and Procedures

Entry into this study will not affect concomitant treatments for ADA-deficiency, such as prophylactic antibiotics or intravenous gamma globulin therapy. However, the patient will be withdrawn from TMP/SMX and/or dapsone by day -2 at least and may not receive these medications until ANC > 500 x 3 consecutive days post nadir (i.e., myeloid engraftment). Use of monthly aerosolized or intravenous pentamidine, monthly oral atovaquone or other appropriate prophylaxis for *Pneumocystis jirovecii* must be started by Day + 30 after cell product infusion.

6.4 Precautionary and Prohibited Medications and Procedures

6.4.1 Prohibited Medications and Procedures

Criteria for PEG-ADA administration

PEG-ADA will be continued through marrow harvest, conditioning and cell product infusion. PEG-ADA will be stopped on day +30 post cell product infusion if the patient received busulfan and the final cell product, and does not have active infections or other major medical problems.

We will recommend restarting PEG-ADA if:

By 12 months after the cell product infusion there is any one of the following:

a) no evidence of PBMC or erythrocyte ADA enzyme activity above baseline/pre-treatment level,

b) no evidence of immune reconstitution (absolute # CD3 cells <200/mm^3),

c) no detectable gene-marked granulocytes by ddPCR/qPCR (<1/10,000 cells).

The patient will be put back on PEG-ADA, and be followed-up medically.

PEG-ADA **may** be re-started if:

The PI deems it in the best interest of the subject on clinical grounds e.g. multiple, serious or unresponsive infections or sub-normal immune reconstitution.

PEG-ADA **may** be re-stopped (after it has been re-started for above reasons) if:

Criteria for being off are present: no active infections, PBMC or erythrocyte ADA >baseline/pre-treatment levels, absolute CD3+ T cells counts ≥200/mm^3, and peripheral blood samples are positive for vector sequences by ddPCR/qPCR.

6.4.2 Precautionary Medications and Procedures

We also prefer for patients to abstain from myelosuppressive medications, including bactrim for *Pneumocystis jirovecii* prophylaxis. However, if medications that fit this description are clinically warranted, and there are no viable alternatives, then the subject may receive the medication.

6.5 Prophylactic Medications and Procedures

Prophylactic medications:

Subjects will receive acetaminophen and diphenhydramine prior to the infusion of OTL-101.

As a part of standard care for autologous hematopoietic stem cell transplantation, patients will be provided coverage for *Pneumocystis jirovecii* and mucosal yeast infection until adequate immune reconstitution has occurred (see Section 7.4). Medications may include trimethoprim/sulfamethoxazole, pentamidine, atovaquone, or other effective PJP prophylaxis started by Day + 30 after cell product infusion and p.o. fluconazole or nystatin for mucosal thrush prophylaxis. Prophylactic anti-viral medications (e.g. acyclovir) may be given for clinical care considerations, but are not required in the absence of evidence of infection or exposure to herpes or other viruses.

Seizures are a rare, but known side effect of busulfan. To prevent this occurrence, we will prophylactically treat our subjects with oral or intravenous Keppra prior to starting busulfan until at least 24 hours after completion. Nausea and vomiting are other side effects of busulfan chemotherapy. Subjects will receive oral or intravenous Ondansetron prior to starting busulfan to prevent nausea and vomiting.

Standard treatment for patients with SCID includes regular immunoglobulin administered either subcutaneously or intravenously. We will continue this through the process. Patients will not receive any other prophylactic medications unless clinically warranted.

6.6 Rescue Medications

Rescue medications may be needed as a part of standard treatment for autologous hematopoietic stem cell transplantation. If the subjects experience fever while neutropenic, they will receive broad spectrum intravenous antimicrobials as per the guidelines at UCLA or NIH. The antimicrobials will be tailored based on clinical status and for any culture sensitivity results. Subjects will receive oral acetaminophen and/or oral/intravenous narcotics for pain that may occur

as a result of the bone marrow harvest. Subjects may have a reaction (e.g. rash, hypertension, hypotension, dyspnea) to the infusion of autologous cells for which they will be treated per the guidelines at UCLA or NIH. Patients may be placed back on PEG-ADA if they fail to achieve immune reconstitution and have unresponsive infections.

7 STUDY PROCEDURES/EVALUATIONS

7.1 OVERALL STUDY PLAN

These studies will seek to determine the safety (as primary end-point) and efficacy (as secondary end-points) of transplantation of OTL-101to provide functional immunity without exogenous enzyme replacement therapy. Overall, the study procedures are within one of four distinct phases:

1. Pre-treatment
2. Treatment
3. Post-treatment
4. Extended Follow-up

The following is a brief overview of the Study Plan. The detailed study plan is described in **Appendix B: Schedule of Procedures/Evaluations**

7.2 Pre-treatment phase:

Baseline Screening: The first part of the study will involve performing laboratory tests to determine whether the subject meets the inclusion criteria and does not have a contra-indication causing exclusion.

Continuation/Cessation of Medications/Procedures: If enrolled into this study and meeting all eligibility criteria, the subject will continue PEG-ADA ERT and prophylactic intravenous or subcutaneous immunoglobulin replacement. Trimethoprim/sulfamethoxazole, if being given, will be stopped 5-10 days prior to bone marrow harvest.

Central venous access: If the subject does not already have a central venous access device, a central venous access device will be placed for ease of phlebotomy and bone marrow cells and drug administration. The decision between percutaneous intravenous central catheter (PICC) line, a tunneled central venous catheter, or an implanted sub-cutaneous access device will be made by discussions with parents and line placement surgeon. A PICC line may instead be placed as a separate procedure with appropriate sedation or a central venous line may be placed during the general anesthesia for the bone marrow harvest.

7.3 Treatment Phase:

Bone marrow harvest: Bone marrow harvest will be under general anesthesia to ≤20 ml/kg.

For patients older than 2 years of age, where our experience indicates that the yield of bone marrow CD34+ cells is lower than in younger children, the patient may be asked to have a "back-up" bone marrow harvest performed approximately 1-3 months prior to the actual gene transfer procedure. The back-up must contain ≥5x10^7 nucleated cells/kg from whole bone marrow or 3x10^7 mononuclear cells from ficolled marrow. Marrow mononuclear cells will be cryopreserved and kept as the back-up.

Back-up bone marrow: A backup bone marrow inoculum (5x10^7nucleated cells/kg from whole marrow or 3x10^7 mononuclear cells from ficolled marrow) will be cryopreserved, and CD34+ cells will be isolated from the remainder.

Cell sufficiency criteria and resulting action: Once the CD34+ cells are isolated and counted, the number of CD34+ cells/kg is calculated.

1) If a sufficient number of cells (≥1 x 10^6/kg CD34+ cells) are isolated after the back-up was removed, the subject will receive busulfan (**See Appendix C**), the CD34+ cells will be transduced with the EFS-ADA lentiviral vector, and OTL-101 will be re-administered if they meet release criteria.

2) If less than 5 x 10^5 CD34+ cells/kg are isolated from the initial harvest, the subject would meet subject stopping criteria, would not receive busulfan, would be withdrawn from the study, would remain on PEG-ADA and would return to routine medical care.

3) If between ≥5 x 10^5 to <1 x 10^6/kg CD34+ cells are isolated, the CD34+ cells will be cryopreserved as additional back-up and PEG-ADA will be continued.

The patient will be offered to return later for a second collection to isolate the cells for the transduction. If >1.0x10^6 CD34+ cells/kg are isolated from a second harvest (and adequate back-up had been cryopreserved from the first harvest), these cells will be transduced with the EFS-ADA lentiviral vector, the subject will receive busulfan, and OTL-101.

4) If OTL-101 fails to meet any one of the release criteria and the subject has received busulfan, the OTL-101 will not be given, the back-up cells will be administered, the subject would meet subject stopping criteria, and s/he would be withdrawn from the study, would remain on PEG-ADA and would return to routine medical care.

5) If ≤ 0.5 x 10^6/kg cells are in OTL-101, which meets release criteria, OTL-101 will be administered and then the back-up cells will also be given within 1-2 days after OTL-101 has been given.

7.4 Post-treatment Phase:

Post-transplant care: Subjects will be kept in the hospital for close monitoring for at least 7 days post-infusion of the cells. However, they may be kept for a longer period as deemed clinically necessary, depending on medical care needed (e.g. CVC care, infections, protective isolation,

nutritional support), consequences of toxicity to Busulfan, engraftment of transduced cells and immune reconstitution. Alternatively, for out of town subjects who are clinically stable, post-transplant care may be transferred to the home physician provided they have agreed to assume the post-transplant monitoring. The monitoring can take place as an in-patient or as an outpatient. In this case, the subject will stay a minimum of 3 days after infusion of the cells at the treatment facility (UCLA or NIH) prior to being transferred. This planned discharge and subsequent re-admission to a second facility would not constitute an adverse event.

PEG-ADA will be stopped on day +30 post-cell product infusion if the patient received busulfan and the final cell product and does not have active infections or other major medical problems.

For subjects who have slow recovery of counts after transplant (beyond Day +30), CBC/Diff will be performed at least once every 7-10 days, until ANC is >500 cells/mm^3. If after day +42 the criteria for non-engraftment are met (persistent ANC < 200/µl or platelets < 20,000/µl) after cell infusion on three independent and consecutive determinations over at least ten days, the back-up cells will be given. If hematologic reconstitution does not occur by Day +90 from the initial infusion of the cell product, prolonged unresponsive pancytopenia will exist.

Infection prophylaxis: The subjects will be maintained on prophylactic antibiotics and intravenous gamma-globulin replacement and will be closely monitored as an outpatient. They will be readmitted to a hospital when deemed clinically necessary. These reasons include, but are not limited to: fever and neutropenia, bacteremia, possible central venous access device infection, or other infections that require initial parenteral antibiotics, organ failure, extreme adverse social situations, and/or bleeding.

Prophylactic antibiotics will be used to provide coverage for *Pneumocystis jirovecii* pneumonia and mucosal yeast:

a) *Pneumocystis jirovecii* prophylaxis (PJP): Trimethoprim/sulfamethoxazole (TMP/SMX) will be stopped prior to marrow harvest and an alternative PJP prophylaxis regimen will be used at least until ANC>500 for three consecutive measurements, when TMP/SMX may be resumed. Suitable alternative PJP prophylaxis medications include pentamidine (intravenous or aerosolized) or atovaquone. Dapsone should be used with caution, due to its potential myelosuppressive effects. PCP prophylaxis may be stopped when CD4>200/ul and PHA response is >50% lower limit of lab normal response range (Griffith, 2009).

b) Patients will be given a prophylactic antifungal medication, such as oral nystatin or fluconazole in standard doses. This may be stopped when CD3>200/ul.

Additional or alternative antibiotics may be administered based on clinical care considerations (these will be recorded as Concomitant Medications).

Intravenous (or SQ) gamma-globulin will be administered at standard dosage and frequency (e.g. 400-600 mg/kg q3-4 weeks). Indications for considering stopping immunoglobulin replacement include: absolute CD4>200/µL, absolute B cell >100/µL, IgA or IgM > LLN for age. Immunoglobulin replacement may be continued based on clinical care considerations.

7.5 Extended Follow-up Phase:

Research Evaluations: After the infusion of the final cell product, follow-up studies will determine whether gene-modified cells have engrafted and are able to produce mature lymphoid and hematopoietic cells containing and expressing the ADA gene and the extent and time-course of immune reconstitution. Serial samples of peripheral blood will be analyzed for the frequency of cells containing the inserted ADA vector over a two year follow-up phase.

Stable persistence of immune function in the absence of PEG-ADA treatment will constitute evidence that the gene transfer has led to clinically beneficial effects on immune function. The Phase I/II study will also attempt to determine whether the presence of mature lymphoid cells will contain and express the EFS-ADA vector in greater frequency than the subjects in the prior γ-retroviral vector trials and if those cells will contribute to more rapid or robust immune function.

If by 6 months after cell product infusion, there is no evidence of PBMC or erythrocyte ADA enzyme activity (over baseline/pre-treatment levels), immune reconstitution (<200/μL absolute CD3+ T) and no detectable ADA gene-marking in granuloctyes by ddPCR/qPCR (<1/100,000), PEG-ADA will be re-started.

Patients will be monitored for clonal expansion, once gene marking levels exceed 1% (see detailed monitoring schedule in **Appendix F**.

Long-term follow-up: After completion of the two years of post-treatment evaluation of this study, patients will be offered enrollment in a long-term follow-up study. We will attempt to maintain annual contact with all treated subjects and will obtain samples of peripheral blood cells and serum annually for archiving at -80°C, to be available for investigation of potential complications related to lentiviral-mediated gene transfer, e.g. to assess the presence of serum antibodies to lentiviral vector proteins, the presence of replication-competent lentivirus in peripheral blood cells or clonal dominance.

7.6 Clinical and Laboratory Evaluations

7.6.1 Clinical and Research Laboratory Evaluations and Specimen Collection

Follow-up:

1. Hematology: hemoglobin, hematocrit, WBC count with differential, platelet count [specimen: 2mL whole blood anti-coagulated with EDTA; method: automated counter (e.g. Coulter counter, or equivalent)]

2. Biochemistry: albumin, creatinine, total bilirubin, ALT, AST, glucose (non-fasting), total protein, calcium, electrolytes, alkaline phosphatase, BUN (specimen: 1mL serum; method: automated machine)

Special Assays

1. Measurements of deoxyadenosine metabolites and ADA activity in RBC (specimen: 2mL whole blood anti-coagulated with heparin, method: per protocol in Hershfield lab at Duke University).

2. Measurement of ADA expression in PBMCs by ADA enzymatic assay (specimen: 4mL whole blood anti-coagulated with heparin, method: ADA enzyme research assay kit, Diazyme).

3. Determination of absolute numbers of CD3+, CD4+ and CD8+ T lymphocytes, CD19+ B lymphocytes and CD56/CD16+ NK cells (specimen: 4mL whole blood anti-coagulated with EDTA; method: flow cytometry); CD4+/CD45RA+ and CD4+/CD45RO sub-sets, if sufficient cells are obtained.

4. T cell proliferative responses to PHA, tetanus toxoid (specimen: 2-8mL whole blood anti-coagulated with heparin, method: ^{3}H incorporation or CFSE labeling)

5. T-cell receptor excision circles (TREC): As a measure of *de novo* T lymphopoiesis, T-cell receptor excision circles (TREC) will be measured in CD3+ PBMC at serial time-points (and CD4 and CD8 + cells, if available). This assay will be performed in the Bone Marrow Lab of Great Ormond Street Hospital (GOSH) for Children, London, UK, again to provide consistency between the US and UK trials.

6. TCR Vβ family usage on CD3+ PBMC by flow cytometric analysis will be performed at the UCLA and NIH Clinical Immunology Labs.

7. Quantitative determination of immunoglobulin (IgG, IgA, IgM) levels (specimen: 0.5mL serum, method: per clinical laboratory)

8. Determination of specific antibodies to tetanus toxoid and phosphoribosyl phosphate (PRP) (~every 3 months if not receiving IVIg; specimen: 0.1mL serum)

9. Measurement of the frequency of cells containing the inserted ADA gene in PBMC and granulocytes, as well as MACS-sorted T cells and myeloid cells (if quantities permit) (specimen: 4mL whole blood anti-coagulated with heparin or EDTA, method: quantitative PCR or digital droplet PCR)

10. Determination of replication competent lentivirus (RCL) at baseline, 3, 6, 12, and 24 months post-infusion and archived annually thereafter (specimen: 2mL whole blood anti-coagulated with heparin; method: DNA PCR at NGVB).

11. Sample banking at baseline, 3, 6, 12, and 24 months post-infusion and annually thereafter, for possible western blot testing (WB) for RCL (specimen: 0.5mL serum, method: WB at NGVB).

12. Leukemia monitoring at baseline, and at ~6-month intervals for the first five years post-infusion, then annually for an additional 10 years (15-year total monitoring after infusion). Performed only if criteria for triggering leukemia determination are fulfilled (see monitoring plan) [specimen: 2mL whole blood anti-coagulated with heparin, method: linear amplification mediated (LAM)-PCR] (at NGVB)

13. **Integration Site Analysis:** To monitor vector integration sites, both for safety and scientific analyses, genomic DNA from PBMC at specific time-points will be analyzed using non-restrictive LAM-PCR and bioinformatic analysis at UCLA and NHGRI.

A general protocol for investigation of possible clonal proliferations that might develop as a result of insertional oncogenesis will be followed. The purposes of the protocol are to:

Provide adequate monitoring so that determination of the need for therapeutic intervention is made as expeditiously as possible;
Minimize the risk of either physical or psychological harm of unnecessary interventions, to which subjects are exposed;
Characterize any clonal proliferation events occurring in gene therapy trials;
Determine whether any clonal proliferation resulted from insertional oncogenesis;
Characterize the clonality of the normal, genetically-modified hematopoiesis after lentiviral gene therapy of HSC.

(A detailed method and protocol are discussed in Appendix F - Monitoring for Monoclonal Expansion or Leukoproliferation.)

Specimen Preparation, Handling and Shipping

Biohazard Containment

The vector supernatant will be shipped on dry ice under UN 2814 (infectious substances) and UN 1845 (dry ice) from IUPVF to UCLA and NIH. The bone marrow harvest will be performed in an Operating Room at the clinical site. These Operating Rooms meet the national standards for the Joint Commission for Accreditation of Healthcare Organizations (JCAHO) and Occupational Safety and Health Administration (OSHA). The cells will then be transported for CD34+ isolation in a closed container for processing in the Good Manufacturing Practice (GMP) suite and the UCLA Bone Marrow-Stem Cell Laboratory or in the Cell Processing Section of the Department of Transfusion Medicine (CPS, DTM) of the NIH Clinical Center. All viral transduction will be performed in the GMP suite, which has its own equipment, including a biosafety cabinet, incubator, and centrifuge, as well as negative pressure airflow. The cells will be washed of all viral particles prior to being placed in a syringe for transport and re-infusion. All needles, blood, blood products, viral products and biohazard waste will be handled, shipped, and disposed by following the rules and regulations set forth by the UCLA Office of Environmental Health and Safety and NIH IBC through recommendations from the Centers for Disease Control and Prevention and the National Institutes of Health. Universal precautions will be used when in contact with the patient. All infectious specimens will be transported using packaging mandated in the Code of Federal Regulations, 42 CFR Part 72.

Instructions for Specimen Storage

The specimens collected for RCL testing at regular time-points will be stored until the 24 month time-point results are available. If there are no detected RCL, then the samples will become the property of the principal investigator. These specimens may be disposed properly, used for research purposes, or kept in storage indefinitely. The long-term study will monitor the status of the subject to the 15-year mark. If at this time, the subject has hematopoiesis and immune function that does not require PEG-ADA replacement, they have been taken off study, or have passed away, then the back-up can be properly disposed, used for research purposes, or kept in storage indefinitely.

Specimen Shipment Preparation, Handling and Storage

The substances covered under this section include the blood specimens that will be sent to us and other labs for the follow-up laboratory evaluations. Clinical specimens (blood) will be shipped under UN 3373 Category B. All products and specimens will be packed according to IATA DGRs according to the appropriate UN category. Personnel who will perform these functions will have certification from the UCLA or NIH Biosafety Offices for shipping biological materials.

8 STUDY SCHEDULE

8.1 Screening

Obtaining signed informed consent

The principal investigator or co-investigators will invite the subject and subject's family to an informal meeting to introduce the research study. Other investigators, research nurses, the study coordinator, etc. on the study may also be present to answer questions. The subject/subject's family will be given a sample copy of the informed consent to take home and read at their leisure. The consent will have the investigator's contact information, if they have questions about the research study.

If the subject/legal representative agrees to participate, the informed consent will take place in a conference room. Before the actual consent meeting, the investigator and subject/representative(s) will have already had informal discussions about the study, and a sample copy of the consent will have been given to the subject/representative(s) to read ahead of time.

At UCLA, consent by teleconference will be obtained in some cases, if the subject and his/her legal representative(s) live outside of Los Angeles, for the benefit of the subject. If this is the case, the consent will take place in an office or conference room designated by the subject's home physician. The home physician will be present with the subject/subject's legal representative(s) during the consent by teleconference between the subject/representative(s) and investigator.

At the informed consent, the consent is reviewed sentence by sentence, with frequent determinations (at least every page) whether there are questions from the potential subject/legal representative(s)/family. Any and all questions will be answered by the investigator(s) to the best of his ability. If the subject is between 7-12 years old, he/she will be asked to read the assent form and allowed to ask questions. A child between 7-12 years old must sign the assent form in order to participate in the research study. Once permission has been given (all applicable consents signed), the screening tests to determine eligibility will be scheduled.

Screening

Screening tests will be performed within 60 days prior to enrollment. We may use test results that already exist at the time the informed consent is obtained, provided that the date of the results fall within the criteria set above.

1. Complete history and physical examination including vital signs (temperature, pulse rate, respiratory rate and blood pressure).
2. Blood tests:
    a) Comprehensive metabolic panel (albumin, AST/ALT, alkaline phosphatase, total protein, total bilirubin, creatinine, BUN, glucose, calcium, Na+, K+, Cl-, CO_2)
    b) magnesium, phosphate
    c) CBC with differential and platelet count
    d) INR or PT/PTT
    e) HIV-1 and hepatitis B, hepatitis C, CMV, and parvovirus B19 by DNA PCR
    f) Peripheral blood for cytogenetic analyses (if cytogenetic testing was not performed on cells from amniocentesis)
    g) Pregnancy test, if female of child-bearing age
3. Urinalysis: routine urine chemistry and microscopic examination
4. Electrocardiogram (ECG)
5. Echocardiogram (Echo)
6. Chest X-ray (CXR)
7. Pulse oximetry
8. Biopsy of suspicious skin lesions
9. Confirmation of ADA deficient SCID*

* Confirmation of ADA-deficient SCID will be based upon biochemical or genetic demonstration of ADA deficiency and T lymphopenia at the time of diagnosis. The date of these results will likely be outside of the parameters set above.

8.2 Enrollment/Baseline

If subjects meet inclusion criteria and have no findings causing exclusion, then subjects will be enrolled.

Physical exam, specified blood tests, and urinalysis will be performed within 3 days of the bone marrow harvest procedure. These studies must meet the inclusion criteria to allow enrolled subjects to undergo the bone marrow harvest. Determinations of ADA enzymatic activity, erythrocyte deoxyadenosine nucleotide levels, and immune function will be performed after screening and before treatment. PBMCs and serum will be collected and banked for future RCL determination.

1. Complete physical examination including recording of height (cm), weight (kg), and vital signs (temperature, pulse rate, respiratory rate and blood pressure).

2. Blood tests:
    a) Comprehensive metabolic panel (albumin, AST/ALT, alkaline phosphatase, total protein, total bilirubin, creatinine, BUN, glucose, calcium, Na+, K+, Cl-, CO_2)
    b) Magnesium, phosphate

c) CBC with differential and platelet count

d) INR or PT/PTT

e) Type and cross for 2 units PRBC

f) Pregnancy test, if female of child-bearing age

3. Urinalysis: routine urine chemistry and microscopic examination.

The following labs must be done prior to infusion of OTL-101:

4. Measurements of leukocyte or erythrocyte ADA enzymatic activity and erythrocyte deoxyadenosine nucleotide levels

5. Measurement of immune function

a. Determination of absolute numbers of CD3, CD4 and CD8 T lymphocytes, CD19 B lymphocytes and CD56/CD16 NK cells.

b. Proliferative responses to PHA, Tetanus toxoid and *Candida*

c. Measurement of serum immunoglobulin levels (IgG, IgA, IgM)

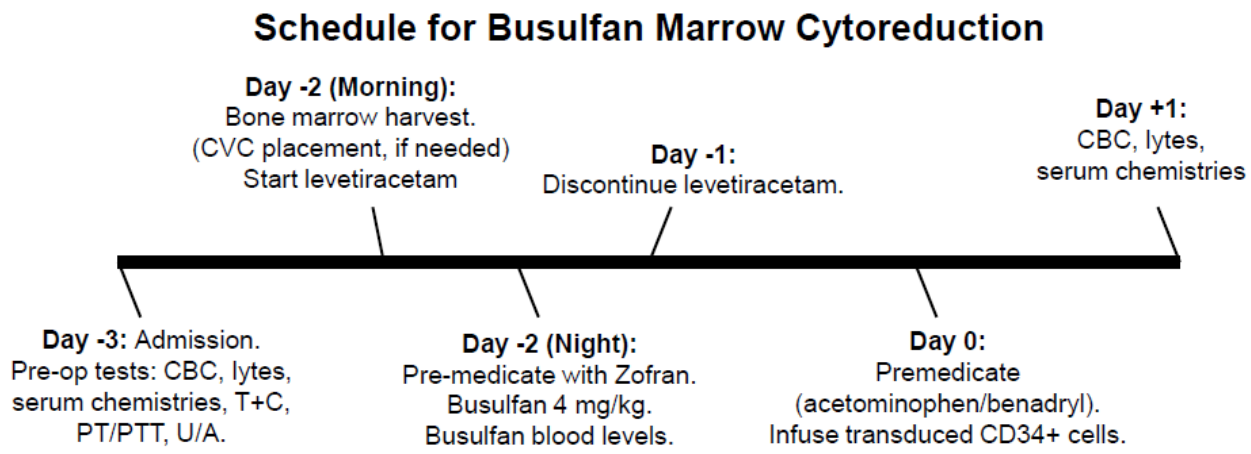
d. Measurement of specific antibodies to tetanus, polyribosylphosphate (PRP)

6. PBMC and serum banking for RCL determination

8.3 Treatment

If physical exam, blood tests, and urinalysis performed within 3 days of the planned bone marrow harvest meet the inclusion criteria, the subjects will undergo the bone marrow harvest under general anesthesia. A total volume of no greater than 20 mL/kg subject body weight will be collected in order to isolate CD34+ hematopoietic progenitor cells. Subjects who meet criteria to receive Busulfan (enough cells to freeze back-up and to start culture with ≥ 1 x 10^6 CD34+ cells/kg) will remain hospitalized after the bone marrow harvest, for busulfan administration.

Busulfan will be administered as a single IV dose (4 mg/kg) over 3 hours approximately 12-24 hours after the bone marrow harvest (See **Appendix C** for Schedule for Busulfan Marrow Cytoreduction). Peripheral blood samples will be drawn at the following time-points for busulfan area-under-the curve (AUC) pharmacokinetic studies: immediately following the completion of the infusion; at 0, 1, 2, 4, 8 and 13 hours after the end of the infusion. These labs may be drawn within 15 minutes of the timepoint, but the actual time in reference to the administration of busulfan should be recorded for accurate AUC calculation.



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If the gene-modified CD34+ cells meet acceptance criteria at the end of the culturing period, they will be infused intravenously into the subject (approximately 24-48 hours after busulfan administration).

8.4 Follow-up

Following the infusion of the gene-modified cells, the subjects will be evaluated and the following exams will be performed (if sufficient samples can be obtained):

Day +1

1. Physical Exam
2. Safety/toxicity studies:
   a) CBC with differential
   b) Serum chemistry panel, electrolytes

Months 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 21, and 24

1. Physical Exam
2. Safety/toxicity studies:
   a) CBC with differential
   b) Serum chemistry panel, electrolytes
   c) PBMC to be analyzed at 3, 6, 12 and 24 months by PCR for RCL and archived annually, thereafter.
   d) Serum to be banked at 3, 6, 12 and 24 months and annually thereafter, for possible western blot testing for RCR.
   e) Monitoring for leukemia (by LAM-PCR) at approximately 6-month intervals for the first five years post-infusion, then annually for an additional 10 years (15-year total monitoring after infusion) if criteria for triggering LAM-PCR are fulfilled (see monitoring plan).
3. Efficacy
   a) Measurements of leukocyte ADA enzymatic activity and deoxyadenosine metabolites in RBC
   b) Immune function testing

i) Determination of absolute numbers of CD3+, CD4+ and CD8+ T lymphocytes CD19+ B lymphocytes and CD56/CD16+ NK cells

ii) Proliferative responses to PHA, tetanus toxoid.

iii) Measurement of serum immunoglobulin levels (IgG, IgA, IgM) (Q 3 months if not receiving IVIg)

iv) Measurement of specific antibodies to tetanus toxoid and phosphoribosyl phosphate

v) TREC

vi) TCR Vβ Usage

4. Gene transduction/expression:

a) Measurement of the frequency of cells containing the inserted ADA gene in PBMC and granulocytes as well as FACS-sorted T cells and myeloid cells by PCR

b) Measurement of ADA expression in leukocytes by ADA enzymatic assay and possibly RT PCR (if samples are positive by DNA-PCR)

c) Vector integration site analysis

The allowable window for the follow-up exams/tests is ± 4 weeks.

After the 24 month time-point, subjects will be enrolled into a separate long-term follow-up protocol, in which they will be followed once every six months for an additional 3 years, and then once per year, for an additional 10 years. The total time that they will be followed after treatment is 15 years (see monitoring plan described in **Appendix F**).

8.5 Final Study Visit

The final visit will occur at year 15 following the infusion of the gene-modified cells. No special procedures or evaluations will be performed at that time. The participant will be given all current contact information of the investigators in case of questions or adverse events possibly related to the protocol.

8.6 Early Termination Visit

Participants may be removed from the study early, if they have no detectable gene marked cells at two consecutive time-points in the second or later years after treatment. No special procedures or evaluations will be performed at this time. The participant will be given all current contact information of the investigators in case of questions.

If voluntary withdrawal occurs post-treatment, the participant will be asked to continue scheduled evaluations, if possible, and asked to seek appropriate care under medical supervision when necessary. All efforts will be made to continue contact with participants.

Where available, limited long-term data (on survival, need for a rescue HSCT or PEG-ADA ERT reinstitution) will be collected for withdrawn patients after their exit from the study, until the end of the expected study follow-up period.

8.7 Pregnancy Visit

It is unlikely that participants will become pregnant while on the study as most of our subjects will be pre-pubertal infants and children. However, follow-up visits will occur as scheduled. Additionally, participants are only given the study agent once (during the treatment phase), so further administration of the study agent is not an issue.

8.8 Unscheduled Visits

If an unscheduled visit is required, a history and physical exam will be performed and laboratory tests will be performed depending on the signs and symptoms related to the unscheduled visit.

9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

Safety is a primary study outcome measure.

9.2 Definition of an Adverse Event (AE)

Any unfavorable and unintended sign, symptom, or disease temporally associated with the use of the investigational agent, without any judgment about causality. Grading of AEs will be defined by an adapted Pediatric Clinical Toxicity Scale from the NIAID, Division of AIDS (see **Appendix G**).

9.3 Definition of a Serious Adverse Event (SAE)

Serious Adverse Event (SAE): A Serious Adverse Event is defined as an AE meeting one of the following conditions:

1. Death during the period of protocol defined surveillance
2. Life Threatening Event (defined as a participant at immediate risk of death at the time of the event)
3. An event requiring inpatient hospitalization or prolongation of existing hospitalization during the period of protocol defined surveillance (NOTE: hospital admission that is due to transfer of care for post-transplant monitoring will not be considered an SAE.)
4. Results in congenital anomaly or birth defect
5. Results in a persistent or significant disability/incapacity

Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

9.4 Methods and Timing for Assessing, Recording, and Analyzing, Managing Safety Parameters

9.4.1 Methods and Timing for Assessment

Following the infusion of the gene-modified cells (study agent), the subject will visit a clinical investigator at the following time points: day 1, months 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 21, and 24. At each time point, a history (solicited) and physical exam and phlebotomy (unsolicited) for lab tests will be performed. The notes of the history and results of the physical and lab tests will be used to determine adverse event. Notes and results will be recorded on case report forms (CRFs). Any physical and medical condition and lab value outside of normal parameters will be noted and compared to the NAIAD Pediatric AIDS Toxicity Table (**Appendix G**) to determine the grade of the abnormal parameter. In years 3-15, subjects will continue to be followed, albeit less often (1-2 times/year), for IO monitoring. The IO monitoring plan is given in **Appendix F**.

9.4.1.1 AE/SAE Grading and Relationship Assignment

Intensity of AEs will be assigned using the NIAID Pediatric AIDS Toxicity Table (**Appendix G**)

Grade 1 (Mild): events require minimal or no treatment and do not interfere with the patient's daily activities.

Grade 2 (Moderate): events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

Grade 3 (Severe): events interrupt a patient's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Grade 4 (Life threatening): Any adverse drug experience that places the patient or participant, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

Grade 5 (Death)

Relationship assessment of AEs to the study agent/intervention will be made by the principal investigator, as follows:

Definitely Related

There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study agent/intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to the intervention should be clinically plausible. The event must be pharmacologically or phenomenologically definitive.

Probably Related

There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time sequence to administration of the study agent/intervention, is unlikely to be attributed to

concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal or study intervention.

Possibly Related

There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g., the subject's clinical condition, other concomitant events). Although an adverse drug event may rate only as "possible" soon after discovery, it can be flagged as requiring more information and later be upgraded to probable or certain as appropriate.

Unlikely

A clinical event, including an abnormal laboratory test result, whose temporal relationship to study agent/intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the subject's clinical condition, other concomitant treatments).

Not related

The AE is completely independent of study agent/intervention administration, and/or evidence exists that the event is definitely related to another etiology. There will be an alternative, definitive etiology documented by the clinician.

Expected

The clinical event has been described in the informed consent, protocol, or I.B.

An expected event related to disease process includes decreased immune function (lymphopenia) possibly leading to infection requiring hospitalization. Hospitalization is considered a Grade 3/4 AE, which may last one to several days.

Unexpected

The clinical event has not been described in the informed consent, protocol, or IB.

9.4.2 Recording/Documentation

AEs are documented on a CRF. The AE CRF will collect the following primary information:

a. diagnosis
b. date of onset
c. maximum toxicity (using DAIDS toxicity scale)
d. outcome
e. date of resolution or death
f. action taken with trial study agent
g. withdrawal of subject from study (if applicable)
h. relatedness to study agent

i. seriousness assessment

Additional information captured on the CRF will include:

a. possible causes of the SAE other than trial medication
b. relevant medical conditions
c. other relevant risk factors
d. trial medication details
e. relevant concurrent medications

Grade 3 or 4 AEs are reported to the PI or study coordinator, as well as to the Sponsor, within 48 hours.

9.4.3 Analysis/Management

The subjects are followed by a clinician who has been taught the criteria for SAE. The CRF includes a column for entering the date of resolution of the AE. The CRF is completed by the study coordinator and reviewed by the PI. The PI decides whether new information on the study (or other related studies) should be communicated to the subjects.

9.5 Reporting Procedures

9.5.1 General Details for Reporting Procedures

- Adverse Events (AEs) Grades 1 and 2
  - Grade 1-2 adverse events will be reported by the clinical investigators to the sponsor (by AE log) within 1 week of the office visit. The sponsor will review and confirm the grade of AE. The clinician will follow the AE until resolution or stability.
- Serious Adverse Events (SAEs) Grades 3-4

  All Grade 3 and 4 events will be reported to the Sponsor within 48 hours regardless of whether the clinical investigator believes that they are drug related. The clinical investigator will be responsible for following the event(s) until resolution or stability. The site PI will report the SAE to the IRB and the institutional IBC (if applicable) within the guidelines dictated by those entities.

  If the sponsor determines that the event meets reporting requirements to the overseeing regulatory committees, the sponsor will notify other investigators and the regulatory committees, including the DSMB, FDA, and RAC, within the guidelines dictated by each agency (see Section 9.5.2).
- Serious Adverse Events (SAEs): Grade 5 (death)
  - The clinical investigator will notify the site PI immediately. The PI will notify the IRB, IBC, and sponsor within 48 hours. The sponsor will notify the appropriate regulatory agencies, including the DSMB, FDA, OBA RAC and DAIT, NIAID within the regulatory limits dictated by those agencies.

- Events that require reporting in an expedited time frame include leukoproliferative disease and death. Leukoproliferation is an SAE of special interest for this product; its development in this trial would have significant implications for other studies in the field and therefore it will be reported in an expedited time-frame.

9.5.2 Specific Serious Adverse Event Requirements

9.5.2.1 PI to Sponsor

The site PI will report all SAEs, regardless of causality, to the sponsor within 48 hours of the event. In the investigator's report to the sponsor, the investigator must include an assessment of causality (definitely related, probably related, possibly related, unlikely related, or not related), to be considered by the Sponsor when determining whether the event constitutes an expedited report to other regulatory agencies. It is the site PI's responsibility to report the event to the institutional IRB and IBC, according to their reporting guidelines.

9.5.2.2 Sponsor to FDA

We will follow the FDA's Safety Reporting Requirements for INDs (21 CFR part 312) for safety reporting.

The sponsor will report, in an IND safety report, only a suspected adverse reaction that is both serious and unexpected. The IND safety report will be reported to the FDA no later than 15 calendar days after the sponsor's receipt of the information. Any unexpected fatal or life-threatening event, associated with the use of the drug, will be reported to the FDA no later than 7 calendar days after the sponsor's receipt of the information. In all IND safety reports, the sponsor will identify all safety reports previously filed with the IND concerning a similar adverse experience and will analyze the significance of the SAR in light of the previous reports.

Other reports: According to the current reporting guidelines (Final Rule on FDA Safety Reporting, September 2010), the sponsor will also report safety information from other sources, including other clinical investigations, animal or *in vitro* studies, scientific literature, and unpublished scientific papers. Increased occurrences of suspected SARs over that listed in the protocol or IB will also be reported.

9.5.2.3 UCLA to DSMB

UCLA will report all SAEs, regardless of causality, to the overseeing DSMB according to their reporting procedures. Serious adverse events will be reported within 10 calendar days after the sponsor's receipt of the information; grade 5 SAEs (death) will be reported within 2 days of the sponsor's receipt of information. In the sponsor's report to the DSMB, an assessment of causality and an analysis of the significance of the SAE in context of the trial will be included.

9.5.2.4 UCLA to RAC

We will follow the *NIH Guidelines* (Appendix M-I-C-4. Safety Reporting, April 2016) when reporting SUSARs to the RAC.

Any SAE that is both unexpected and associated with the use of the gene transfer product will be reported to the RAC within 15 calendar days after the sponsor's initial receipt of the information.

Any SAE that is fatal or life-threatening, unexpected and associated with use of the gene transfer product will be reported to the RAC within 7 calendar days after the sponsor's initial receipt of the information.

All reportable SAEs will be followed until satisfactory resolution or until the clinical investigator deems the event to be chronic or the participant to be stable.

Anticipated SAEs that are not considered expedited reportable events include:

- Anemia from the bone marrow harvest
- Thrombocytopenia, neutropenia, and elevated liver enzymes from busulfan

All SAEs designated as "not related" to the investigational product under study or to the study intervention and all non-serious AEs will be recorded and reported to the regulatory agencies in the sponsor's annual report to these agencies.

9.6 Reporting Pregnancy

The subjects in this study are infants and children, and thus, pregnancy would not apply to them. For adolescents in whom preventive measures fail, we will report pregnancies to the DSMB, FDA, IRB, ISPRC, NIAID, and RAC. The treatment period is a confined period within 3 days and a HCG is required within 30 days of initiating the procedure. Even if there should b the unlikely event of pregnancy, there would not be any further administration of study agents.

9.7 Type and Duration of the Follow-up of Participants after Adverse Events

Before the subject leaves the office or hospital, a new appointment will be scheduled to follow the AE until the AE resolves or is stable. AEs that are consistent with the procedure/evaluation will be noted on CRFs and re-evaluated at the next follow-up appointment (1-3 months).

9.8 Modification of Study Agent(s)/Intervention(s) for a Participant

This trial involves a single administration of autologous cells and no modifications are planned.

9.8.1 Dose / Schedule Modifications for a Participant

No dose/schedule modifications will be made.

9.9 Halting Rules for the Protocol

If any one of the following five instances occurs, new enrollment to the protocol or treatment of subjects will be halted, pending investigation of the cause. Findings will be discussed with the FDA, IRB and DSMB and each must agree to conditions by which the study can be resumed before enrolling the next patient. Evaluations of study endpoints of subjects already enrolled and who received the final cell product will continue to be performed.

1. If there is one death or four grade 4 toxicities (except grade IV busulfan-related hematologic serious adverse events {transient leukopenia, anemia, thrombocytopenia resolving within 42 days of transplant} which are anticipated and will be reported, but will not be considered stopping

criteria). Additionally, neutropenia (a documented disorder of ADA-deficiency) occurring at any time, will be graded for severity but will not count towards the stopping rule.

2. If two subjects have prolonged unresponsive pancytopenia. This is defined as initial failure at hematologic reconstitution which does not improve following administration of the back-up marrow. Failure of hematologic reconstitution is defined as at least any two of the following being: ANC <200/mm^3, and/or platelets < 20,000/mm^3 without transfusions, and/or Hb < 8.0 gm/dl without transfusions) on three independent and consecutive determinations over at least ten days beyond day +42 from initial infusion of the cell product. If these conditions are reached, the back-up marrow will be infused IV. If failure of hematologic reconstitution persist after the back-up marrow has been given through day +90 from the initial infusion of the cell product, prolonged unresponsive pancytopenia will exist.

3. If 0 of 3 successive patients show evidence of engraftment of transduced cells by six months of follow-up (all peripheral blood samples negative by ddPCR/qPCR for vector sequences (<1/10,000) or by ADA enzyme assay).

4. If RCL is detected, and confirmed in one subject.

5. If a subject in this study develops hematological proliferative, monoclonal expansion or malignant disease (excluding DFSP). A thorough investigation of the cause of the proliferation, expansion or malignancy, including proviral integration analysis, will be done.

9.10 Stopping Rules for an Individual Participant/Cohort

Participants will be removed from study if an adequate cell product cannot be produced (inadequate cell number or failure of cell product to meet release criteria).

Participants may be removed from the study, if they have no detectable gene marked cells (<1/10,000) at two consecutive time-points six months or later after cell product infusion.

No special procedures or evaluations will be performed at the time of withdrawal from the study. Patients will resume standard medical care for their underlying disease and/or receive other treatments at the decision of the patient and primary physician. The participant will be given all current contact information of the investigators in case of questions. Where available, limited long-term data (on survival, need for a rescue HSCT or PEG-ADA ERT reinstitution) will be collected for withdrawn patients after their exit from the study, until the end of the expected study follow-up period.

9.11 Premature Withdrawal of a Participant

If voluntary withdrawal occurs post-treatment, the participant will be asked to continue scheduled evaluations, if possible, and asked to seek appropriate care under medical supervision when necessary. All efforts will be made to continue contact with participants. In addition, efforts will be made to obtain consent for use of the data collected to the time of withdrawal, but it will be determined by the subject or their representative. Where available, limited long-term data (on

survival, need for a rescue HSCT or PEG-ADA ERT reinstitution) will be collected for withdrawn patients after their exit from the study, until the end of the expected study follow-up period.

9.12 Replacement of a Participant Who Discontinues Study Treatment

Subjects who discontinue study prior to infusion of gene-modified cells will be replaced. A total of 20 subjects will be enrolled.

10 CLINICAL MONITORING STRUCTURE

10.1 Site Monitoring Plan

Overview

- The monitor will be chosen based on qualifications and experience.
- The monitor will be familiar with the study agent, the protocol, laboratory SOPs, informed consent, AEs, and regulatory reporting requirements.
- The duties of the monitor include, but are not limited to ensuring that: investigators are qualified; testing facilities are qualified, study reagents are received, stored, and used properly; adherence to protocol; CRFs are completed appropriately, AEs are handled properly, protocol amendments are submitted properly and communicated to investigators, protocol deviations are documented and corrected; findings are communicated to the PI/sponsor.
- Refer to UCLA Clinical Gene Therapy Laboratory SOP 9301 for detailed procedure.

10.2 Safety Monitoring Plan

The NHLBI Gene and Cell Therapy Data Safety Monitoring Board provided oversight for the conduct of this trial from July 2013 to September 2016. Safety monitoring oversight was transferred to the UCLA Clinical and Translational Science Institute (CTSI) DSMB in September 2016.

10.2.1 Safety Review Plan by the DSMB / SMC

Reports will be submitted to the DSMB using their established guidelines, which will include latest protocol version, confirmation of RAC, IBC, and IRB approval, enrollment numbers, update of subjects, status of the study, and timelines.

11 STATISTICAL CONSIDERATIONS

11.1 Background

The objectives of the statistical analyses described in this section are to demonstrate the safety and efficacy of the fresh formulation of OTL-101, an autologous, genetically modified CD34+ Hematopoietic Stem Cells (HSC) for the treatment of severe combined immunodeficiency due to

adenosine deaminase deficiency (ADA-SCID). Full details of the planned statistical analyses for this study are found in the statistical analysis plan.

11.2 Design Considerations and Data

This is a subject-level historically controlled, prospective, non-randomized Phase I/II clinical trial to assess the safety and efficacy of autologous transplantation of CD34+ cells originated from the bone marrow of ADA-SCID infants and children following human ADA cDNA transfer by the EFS-ADA lentiviral vector.

Descriptive results will be presented for the study cohort.

Comparisons will be made to three cohorts:

1) A historical control group consisting of ADA-SCID HSCT-treated subjects lacking a medically eligible HLA-matched sibling/family donor and treated at GOSH/UCL (UK) or Duke University Children's Hospital, North Carolina Transplant Center (USA) from the year 2000 onward
2) A historical control group consisting of ADA-SCID HSCT-treated subjects having a medically eligible HLA-matched sibling/family donor and treated at GOSH/UCL (UK) or Duke University Children's Hospital, North Carolina Transplant Center (USA) from the year 2000 onward
3) All HSCT-treated subjects from GOSH/UCL or Duke University Children's Hospital, who were treated with HSCT from the year 2000 onward, with any type of donor

Details on the historical HSCT control groups from GOSH/UCL and Duke University Children's Hospital can be found in Appendix H.

Statistical analyses will be performed, and their outcomes presented, in three stages:

- Interim look – 6 months
    - Descriptive statistics for all available subjects
- Primary analysis - Comparison on primary efficacy endpoints to the HSCT control cohorts—12 month follow-up analysis
    - Descriptive statistics for the complete study cohort, with all patients having reached at least 12 month follow-up
    - Comparison to HSCT control cohorts on safety, primary efficacy endpoints and need of immunoglobulin replacement (IgRT) (main secondary efficacy endpoint).
- End of study analysis:
    - Descriptive statistics for the complete study cohort, with all patients having completed the study (24-month follow-up)
    - Comparison to HSCT control cohorts on safety, primary efficacy endpoints and need of immunoglobulin Replacement (IgRT) (main secondary efficacy endpoint).

In each study stage and for all analyses, all relevant data available will be used.

11.3 Endpoints

Study endpoints are provided in Section 4.2. A brief summary of study endpoints is provided below.

11.3.1 Safety

Safety endpoints in this study are AEs, including SAEs, with intensity/severity graded by Common Terminology Criteria for Adverse Events (CTCAE) criteria, including relation to treatment. Special attention will be paid to the following AE types:

- Clinical toxicities
- Exposure to replication-competent lentivirus (RCL)
- Development of monoclonal expansion or leukoproliferative complications from vector insertional effects.

11.3.2 Primary Efficacy

The primary efficacy endpoints in this trial are OS and EvFS at 12 months.

11.3.3 Secondary Efficacy

The secondary efficacy endpoints in this trial fall under the following outcome categories

- Overal survival and event free survival at 24 months
- Efficacy of gene transfer/engraftment of HSC
- ADA expression, enzyme activity and detoxification
- Effects of ADA reconstitution on immune phenotype and function – note that need of IgRT will be assessed under this category
- Immune reconstitution
- Infection rates

11.4 Sample Size Considerations

Due to the ultra-orphan nature of this indication, the number of subjects for this trial is planned based on practical considerations. Specifically, the number of ADA-SCID children meeting eligibility criteria in the catchment area is expected to be 4-5 per year. Thus, N = 20 subjects is a reasonable enrollment number for a completion of the trial in a reasonable timeframe.

It should be noted that N = 20 will provide precision of 7% for 100% success (survival) at any particular time point and 12% precision if one failure is observed in the 20 subjects. Precision is here defined as the half-width of the two-sided 95% CI using the Exact Binomial computations.

In summary, 20 subjects receiving OTL-101 will participate in this trial.

11.5 Analysis Sets

11.5.1 Safety Analysis Set

The safety analysis set will consist of all patients treated with OTL-101 at UCLA/NIH (current study cohort) and the complete HSCT historical control cohort, consisting of ADA-SCID subjects with any type of donor treated with HSCT at either GOSH/UCL (UK) or Duke University Children's Hospital (USA) from the year 2000 onward.

11.5.2 Full Analysis Set

The full analysis set will consist of all patients treated with OTL-101 at UCLA/NIH (current study cohort).

The primary analysis set for efficacy from the HSCT historical control cohort will consist of ADA-SCID patients without a medically eligible HLA-matched sibling/family donor and treated with HSCT at either GOSH/UCL or Duke University Children's Hospital from the year 2000 onwards.

Additional efficacy comparisons will be made with:

- ADA-SCID patients with matched related donors treated with HSCT at either GOSH/UCL or Duke University Children's Hospital from the year 2000 onwards;
- the complete HSCT historical control cohort consisting of ADA-SCID patients with any type of donor treated with HSCT at either GOSH/UCL or Duke University Children's Hospital from the year 2000 onwards.

Note however that for the analysis of primary efficacy, full EFS data for HSCT subjects might only be available for the 12-month (primary) and 24-month (end of study) analyses.

11.6 Statistical Methods

11.6.1 Overview

As noted in Section 11.2, the statistical analysis of this data will be done in three stages, which are presented below in Sections 11.6.2, 11.6.3 and 11.6.4. Since all analyses repeat themselves in the different stages, each will be described in detail the first time it appears, which will be referred to in subsequent sections where it is repeated.

The data will be summarized in tables displaying the mean, standard deviation, median, minimum, maximum and number of subjects per group for continuous data (e.g. age, weight) or in tables displaying count and percentage for categorical data (e.g. gender, previous treatment). Data will be presented by visit, if applicable. Data listing by subject will be provided and, where applicable, presented graphically.

Statistical analyses will be performed and data appendices created with SAS® V9.4 or higher.

11.6.2 Interim Look

The interim look will include subjects from UCLA OTL-101 (all subjects followed for at least 6 months) and aggregate data from HSCT treated subjects treated at GOSH (N ~ 25). The following sections describe analyses for both groups, although EvFS and most secondary endpoints may not be available for the HSCT historical controls at this stage of the analysis.

11.6.2.1 Subject Disposition

Subject disposition will be done on the safety analysis set, with disposition provided by UCLA, and all HSCT subject groups. Subject disposition will be tabulated; the number of enrolled, exposed, prematurely terminated, ongoing and completed subjects will be summarized. A list of dropouts will be prepared including treatment received, reason for discontinuation, and time of discontinuation.

11.6.2.2 Baseline Characteristics

Baseline characteristics will be done on the safety analysis set, with characteristics presented by UCLA, and all HSCT subject cohorts. The following information collected at baseline will be presented:

- Demographics (Age, Gender, etc.)
- Method of diagnosis
- Whether received previous HSCT including: date, transplant cells, donor type and outcome
- Whether received previously PEG-ADA. If yes, for how long
- Whether received previously or is receiving currently immunoglobulin replacement therapy
- Listing of relevant medical history, including infections

11.6.2.3 Safety Analyses

Safety will be analyzed descriptively by arm over time for the safety population. Coding will be done using current MedDRA version or higher. AEs, including SAE's will be presented by:

- System organ class and preferred term

Where appropriate, shift tables will be presented for laboratory parameters, vital signs and physical examination.

11.6.2.4 Efficacy Analyses

11.6.2.4.1 Primary Efficacy Analyses

The primary efficacy endpoints, overall survival (OS) and event free survival (EvFS), will be analyzed descriptively, including frequencies for each type of event for EvFS. Additionally, the following analyses will be done to present and compare the treatment arms, OTL-101 subject and HSCT historical control groups, so as to provide as complete a picture as possible of efficacy given the small sample size:

1. OS and EvFS will be described using Kaplan-Meier curves for time to death/event bytreatment arm, and a comparison between arms made using the log-rank test.

2. OS and EvFS rates at 12 months (for those subjects who have reached the 12-month time point) will be described by arm and the difference between arms presented along with associated exact binomial CIs.

11.6.2.4.2 Secondary Efficacy Analyses

Main Secondary Endpoint

- Overall survival and event free survival at 24 months
- Use of immunoglobulin replacement therapy.

Additional Secondary Endpoints

The following will be presented for the infection endpoint:

- Frequency table for number and percent of patients who experienced 0, 1, 2, or at least 3 infections,
- Rate of infection will be calculated on the basis of person-years.

11.6.3 Primary Analysis

The primary analysis will include data from the current study where all subjects have reached the 12-month time point, HSCT historical controls (with or without HLA-matched related sibling donor, evaluated separately and together).

The following analyses will be repeated, as described in preceding sections:

- Subject disposition (Section 11.6.2.1)
- Baseline characteristics (Section 11.6.2.2)
- Safety (Section 11.6.2.3)
- Efficacy (Section 11.6.2.4)

11.6.4 End of study Analysis

The end of study analysis will repeat the analyses for the preceding section (Section 11.6.3) for the study cohort in which all subjects are expected to have completed 24 month follow-up.

11.7 Safety Review

11.7.1 Stopping Rules for the Protocol

If any of the following five instances occur, the protocol will be stopped, pending investigation of the cause. Findings will be discussed with the FDA, IRB and DSMB before enrolling the next patient. Study endpoints of subjects already enrolled will continue to be obtained.

1. If there is one death, or four grade 4 toxicities, not Busulfan-related (transient leukopenia, anemia, thrombocytopenia resolving within 42 days of transplant, which are anticipated and will be reported, will not be considered stopping criteria). Additionally, neutropenia (a

documented disorder of ADA-deficiency) occurring at any time, will be graded for severity but will not count towards the stopping rule.

2. If two subjects have prolonged pancytopenia, defined as at least any two of the following (ANC < 200mm3, and/or platelets < 20,000mm^3 without transfusions, and/or Hb < 8.0 g/dL without transfusions) on three independent and consecutive determinations over at least ten days beyond day +42 after CD34+ cell infusion.
3. If 0 of 3 successive patients show evidence of engraftment of transduced cells by six months of follow-up (all peripheral blood samples negative by PCR for vector sequences or by ADA enzyme assay).
4. If RCL is detected, and confirmed, in one subject.
5. If a subject in this study develops hematological proliferative, monoclonal expansion or malignant disease (excluding DFSP), active enrollment of further subjects will be placed on hold. A thorough investigation of the cause of the proliferation, expansion or malignancy, including proviral integration analyses, will be done.

11.7.2 Stopping Rules for an Individual Participant/Cohort

This is a Phase I/II Clinical Trial for which there is a single cohort. Participants may be removed from the study early if they have no detectable gene marked cells at two consecutive time-points in the second or later years after treatment. No special procedures or evaluations will be performed at this time. The participant will be given all current contact information of the investigators in case of questions.

11.7.3 Immunogenicity or Efficacy Review

1. Safety (absence of grade III-IV SAE)
2. Overall/event-free survival (survival without need for HSCT or PEG-ADA ERT)
3. Gene-marking by lineage
4. Erythrocyte ADA enzyme activity
5. Absolute lymphocytes on CBC
6. Absolute number T, B, NK lymphocytes in peripheral blood
7. Lymphocyte proliferation to mitogen (PHA) and antigens (tetanus toxoid)
8. Quantitative immunoglobulins by class (IgG, IgA, IgM)
9. Antibody responses [isohemagglutinins, tetanus toxoid, polyribosylphosphate (PRP)]

12 QUALITY CONTROL AND QUALITY ASSURANCE

We will have SOPs for laboratory procedures, training of staff, equipment preventive maintenance, data collection, site monitoring, and audits. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements. The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

The study director will implement quality control procedures beginning with the collection of data on CRFs. CRFs will be checked monthly, and any missing data or data anomalies will be communicated to the site(s) for clarification/resolution. This trial will be performed at both UCLA and the NHGRI/NIH, and all QC/QA requirements will apply. Audits will be conducted at least annually.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 Institutional Review Board/Ethics Committee

This clinical investigation will not begin until an IND is in effect.

The Institutional Review Board (IRB) of the Office for Protection of Research Subjects (OPRS) at the University of California, Los Angeles (UCLA), which holds a current FWA, will be responsible for the initial and continuing review and approval of this study. Any proposed changes will be reported to the IRB.

The investigation will be conducted in accordance with all other applicable regulatory requirements for GCP.

13.2 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the participants and their families. Consent forms describing in detail the Study Agent(s)/Intervention(s) study procedures and risks are given to the participant and written documentation of informed consent is required prior to starting study agent/intervention. Consent forms will be IRB approved and the participant will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the participant and answer any questions that may arise. The participants will sign the informed consent document prior to any procedures being done specifically for the study. The participants will have sufficient opportunity to discuss the study and process the information in the consent process prior to agreeing to participate. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not

be adversely affected if they decline to participate in this study. We may also conduct the informed consent by teleconference with those individuals who live too far away to conveniently travel to UCLA or NIH, where the study will be conducted. In this case, the informed consent discussion will take place in the office of the individual's personal physician with the physician present.

13.2.1 Assent or Informed Consent Process (in Case of a Minor)

Assent will be obtained from individuals between 7-12 years old.

13.3 Exclusion of Women, Minorities, and Children (Special Populations)

Women, minorities and children will be included.

13.4 Participant Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating subjects. The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party, without prior written approval of the sponsor.

The study monitor or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records (office, clinic or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

All documents and records with identifying information (e.g. name, DOB, medical record number, etc.) will be kept in locked cabinets, and only authorized personnel will have access. Records will be kept indefinitely, but for a minimum of 2 years following the discontinuation of the study for clinical development. Data that are presented at meetings or conferences will use personal identification numbers (PIN) in lieu of actual names.

13.5 Study Discontinuation

If the study is discontinued, no additional subjects will be recruited or treated. Patients already treated will be followed by physicians at the responsible institutions. There is not a placebo arm of the study.

14 DATA HANDLING AND RECORD KEEPING

14.1 Data Management Responsibilities

Following each scheduled post-infusion visit, the clinician sends the source documents history, physical examination, and available laboratory reports to the site coordinator. The site coordinator at each site accumulates all source documents (including other lab reports) and completes the CRFs by entering data into the web-based electronic CFRs. The site PI or designated monitor reviews the CRFs against the source documents for accuracy. The source documents from the NIH site (history, physical examination, laboratory reports) are scanned and uploaded into the web-based data repository. At the UCLA site, paper print-outs are made of all transmitted source documents and they are filed in a folder for each subject as a secondary back-up; source documents from UCLA subjects are similarly stored as paper files. The NIH site retains all original signed documents in its files. AEs will be handled as described in Data Safety Monitoring Plan. At the end of the study, CRFs and source documents will be kept indefinitely, but for a minimum of 2 years following the discontinuation of the study for clinical development. All data are collected by the sponsor (Orchard Therapeutics, Ltd.), who oversees the compilation of data for archiving and interpretation

The Protocol Monitor will perform 100% source document verification compared to the data in the Electronic data Base at least every 6-8 weeks. The Program Manager or designated person will visit the NIH site prior to initiation of the trial and at least annually, while the site is open for enrollment, to oversee operations and data management at the site, as described in SOP #9301.03 - CLINICAL TRIAL MONITORING. If the site is closed to enrollment, or has enrolled less than 3 subjects, sponsor monitoring may occur remotely using the electronic database and e-mail to resolve discrepancies/deficiencies. An auditor from the UCLA Clinical and Translational Science Institute (CTSI) will perform an audit of the trial documents maintained by UCLA, on a yearly basis. The monitor/auditor will report their findings to UCLA and CTSI, and UCLA will submit the findings to the UCLA IRB.

14.2 Data Capture Methods

Electronic CRFs will be used to collect data. SIStat will assist the investigators in this study with all aspects of data management, quality assurance, security and IT support and, in particular, electronic CRF forms/AE tracking. They will also build a customized web-site with public and private portals to facilitate dissemination of data and software, as well as research.

The same CRFs will be used at both sites (UCLA and NIH) as web-based electronic data capture forms. The data manager or designated person from each site will deposit the data into SIStat using their own log-in ID, within 1 month of the subject's scheduled follow-up visit, to allow time for completion of tests, such as lymphocyte proliferation assays (which may take up to 2-3 weeks to complete). Copies of source documents from the NIH may be requested to verify deposited data.

All paper documents and records with patient identifying information (e.g. name, DOB, medical record number, etc.) will be kept in locked cabinets, and only authorized personnel will have access. Data will be stored electronically via SIStat secure servers at UCLA that feature a hierarchical system of password protected logins, 128 bit SSL, the secure socket technology used for sensitive transactions on the web and failsafe backup systems. All computers are password protected and only authorized trial personnel have access. Records will be kept indefinitely following the close of the study. Data that are presented at meetings or conferences will use

personal identification numbers (PIN) in lieu of actual names and no other patient specific medical information will be released.

After each follow-up visit, source documents will be collected and used to complete the CRFs for the trial (See Data Capture Methods). Data will be reviewed quarterly by the Clinical Trial Monitoring Group to oversee compliance and safety and to produce scheduled and *ad hoc* reports to DSMB and other oversight entities.

14.3 Types of Data

Types of data collected will include:

a) Medical records that will be maintained on each subject, including medical history, physical examination reports and laboratory results obtained by referring physicians prior to enrollment or during follow-up, as well as similar information obtained by the clinical site investigators in the course of this study

b) Laboratory tests for the trial end-points, including those assessing safety (CBC, chemistry panel, RCL assays, insertional oncogenesis), and efficacy end-points outcome measure (ADA enzyme activity, adenine nucleotide levels, gene marking, clonal diversity immune function).

c) Records of investigational materials (receiving, storage, use, shipping and disposal) will also be maintained at each site.

d) Batch records for each clinical cell processing event.

14.4 Source documents and Access to Source Data/Documents

Source documents for subjects enrolled at a clinical site will be kept at that clinical site. Only authorized clinical trial personnel (study coordinators, study director, monitor, PI, etc.) will have access to source data/documents. All documents and records with protected health information (e.g. name, DOB, medical record number, SSN, etc.) will be kept in locked cabinets, and only authorized personnel will have access. All computers are password protected and only authorized trial personnel have access.

Reports to regulatory entities (IRB, FDA, DSMB, NIAID) or public presentations will not contain protected health information (names, DOB, addresses, SSN, etc), but will refer to subjects using anonymous patient identification numbers (PIN) *in lieu* of actual names and no other patient-specific protected health information will be released. Authorized representative from regulatory agencies (FDA, IRB, DSMB and the funding NIAID) will be granted access to records for the purposes of QA, reviews, audits, safety evaluations, and study progress.

14.5 Timing/Reports

Data and reports are reviewed at least every 1-3 weeks at meetings with trial personnel at the coordinating site. Joint teleconferences between UCLA and the NIH will be held once every 1 or 2 months (as schedules allow). Data from CRFs are compiled quarterly for analysis by the

Biostatistics group, and these analyses are reviewed by the Sponsor and trial biostatistician quarterly to monitor for stopping rule criteria.

Routine reports to regulatory agencies are periodic: biannually to the DSMB (based on their schedule) and yearly to the IRB, FDA, OBA RAC and DAIT, NIAID. Final study reports will be generated at the completion of the study. No data is masked and data is coded only for the purposes of presenting the study to the public. A master list, which is only accessible to study personnel, will be kept to match the individuals to their coded PIN.

14.6 Study Records Retention

At the end of the study, CRFs, source documents, and investigational product records will be kept for a minimum of 2 years. After two years, records will not be disposed without the Sponsor's authorization. At the end of the study, a copy of all datasets will be provided to NIAID by the protocol Monitor or manager on behalf of the Sponsor.

14.7 Protocol Deviations

A protocol deviation is any non-compliance with the clinical trial protocol, the Manual of Operations, or the sponsor's Investigator's Brochure. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. Non-compliance events may be noted by any trial personnel, either during the conduct of trial activities or during review of records. As a result of deviations, corrective actions are to be developed by the site and implemented promptly, including filling out prepared worksheets detailing the violation and the corrective action that was taken, and notifying the sponsor within a timely manner. It will be the responsibility of the site to use continuous vigilance to identify and report deviations according to the guidelines of the IND sponsor. Protocol deviations will be reported to the local IRB per their guidelines and in the annual reports to FDA and NIH RAC. The site PI/study staff will be responsible for knowing and adhering to their IRB requirements.

15 PUBLICATION POLICY

Publication of the results of this trial will be governed by publication policies of the funding institutes. Any presentation, abstract, or manuscript will be made available for review by the NIH supporters prior to submission.

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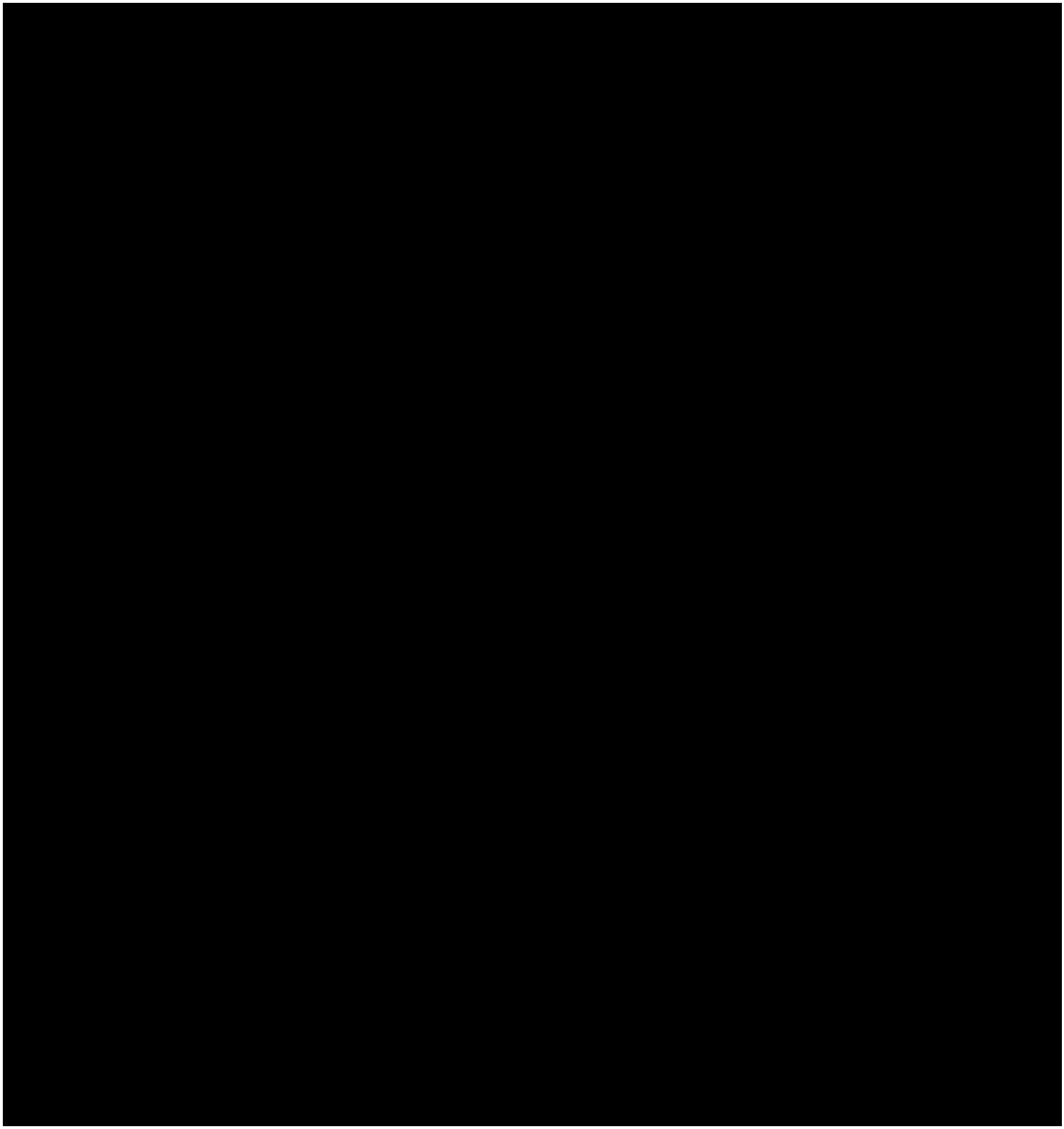
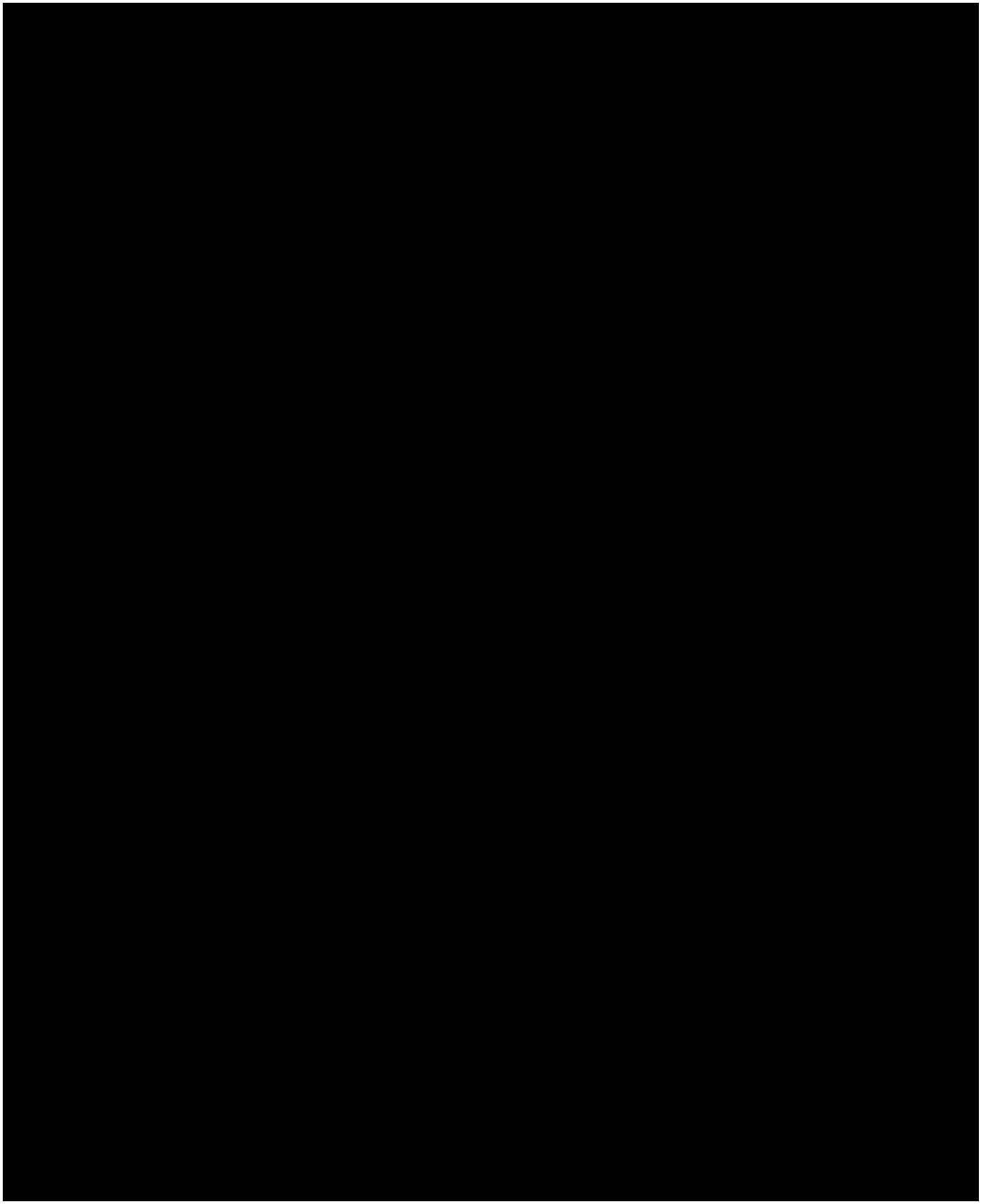
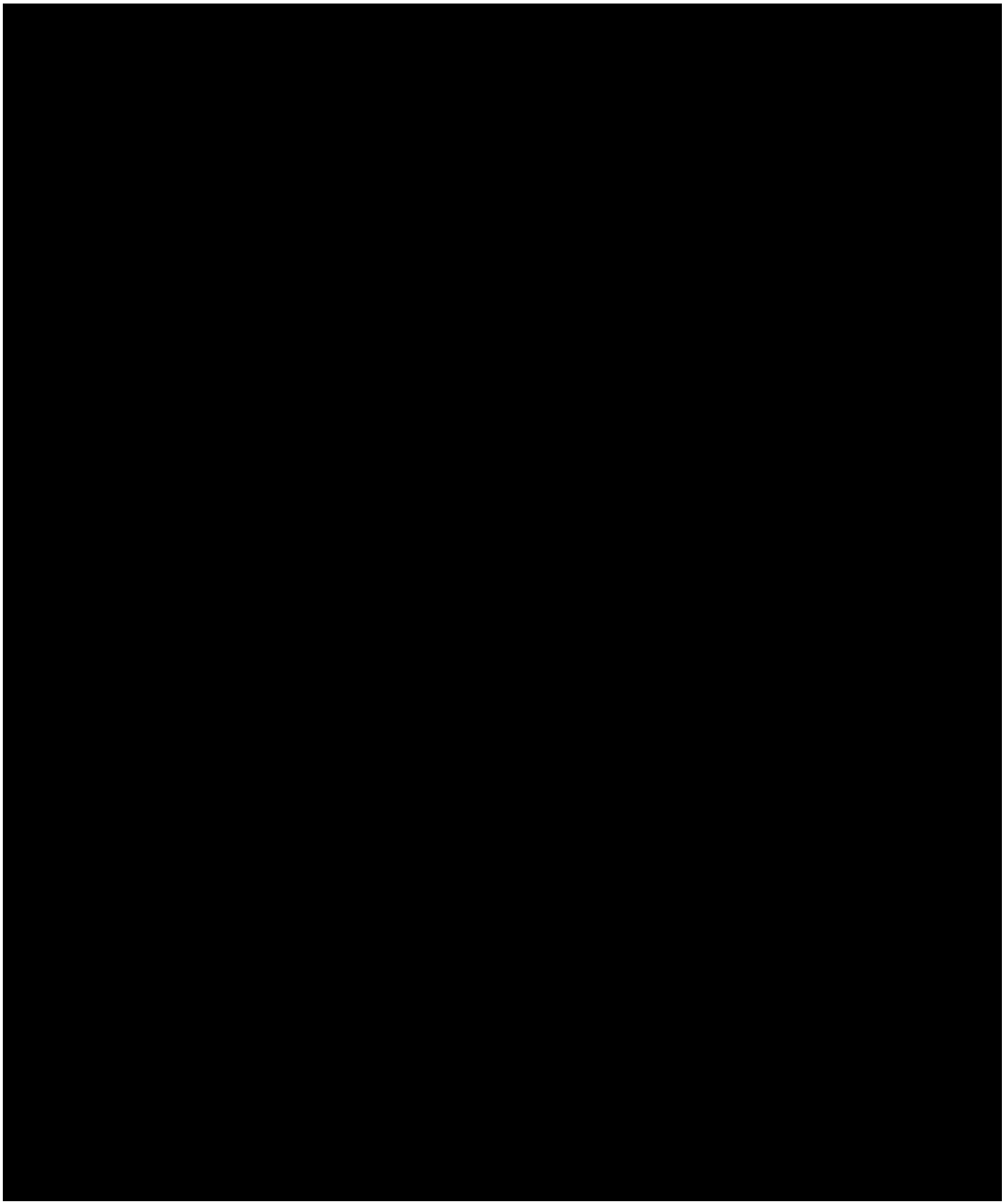
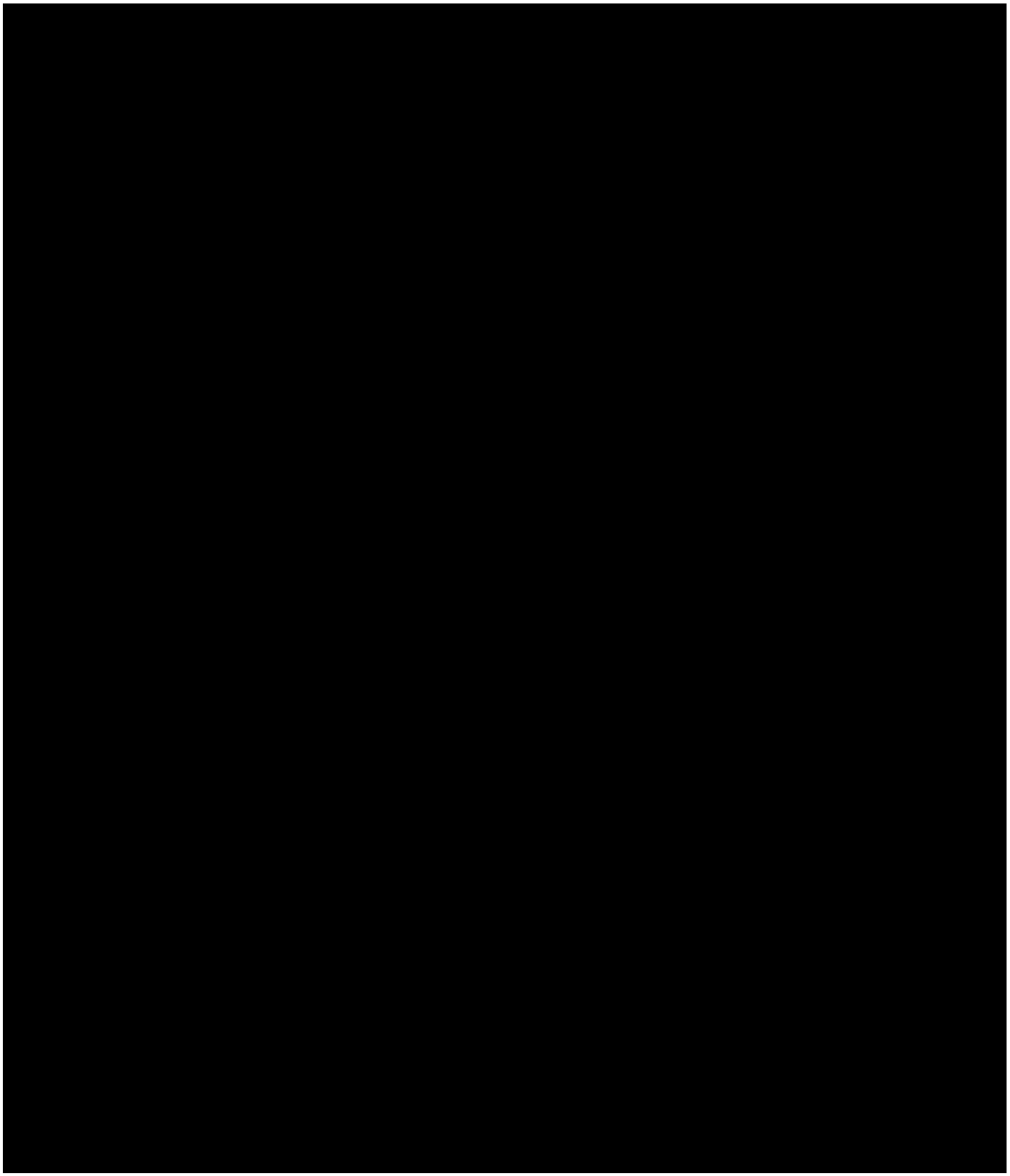
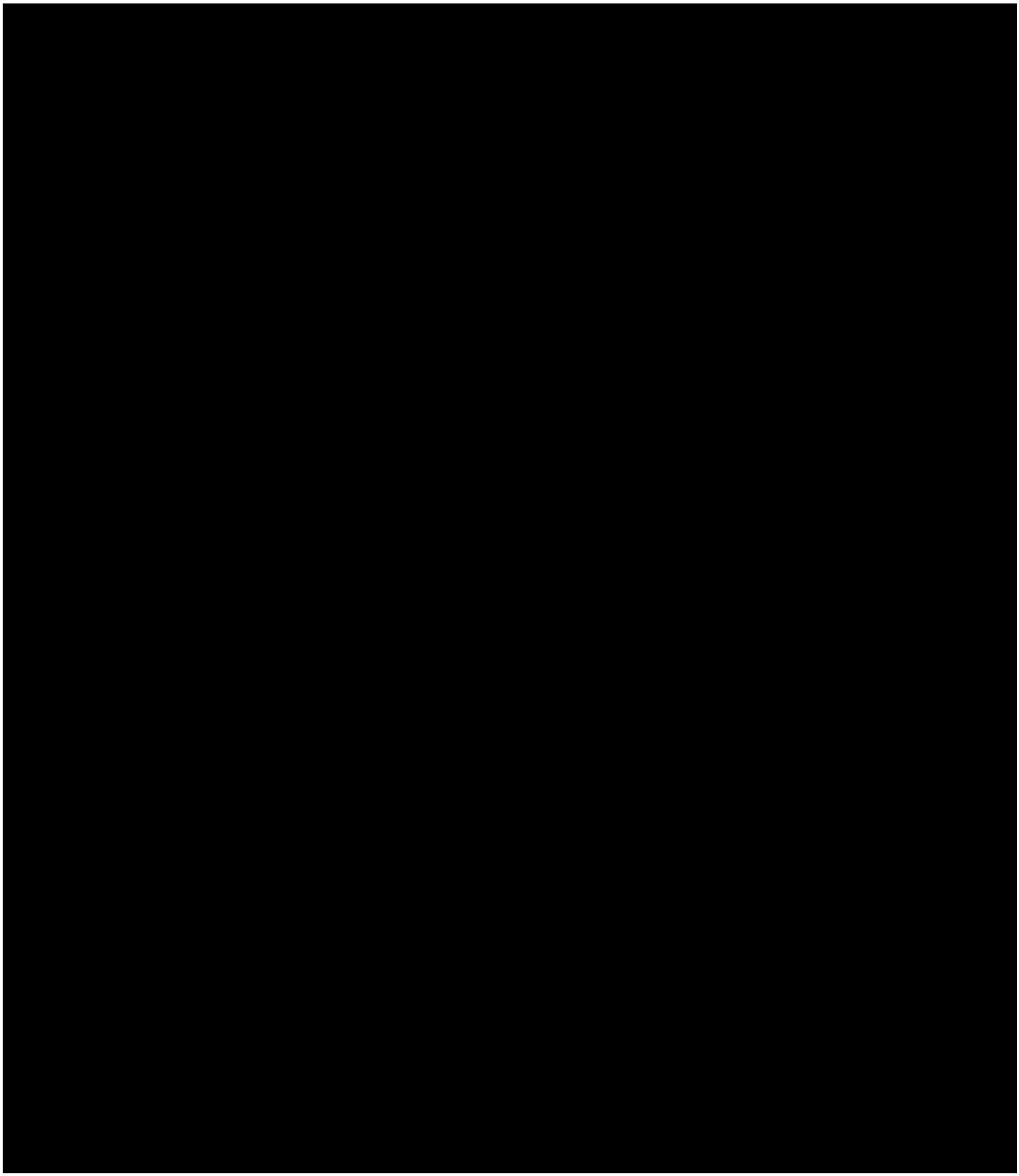
17 APPENDICES

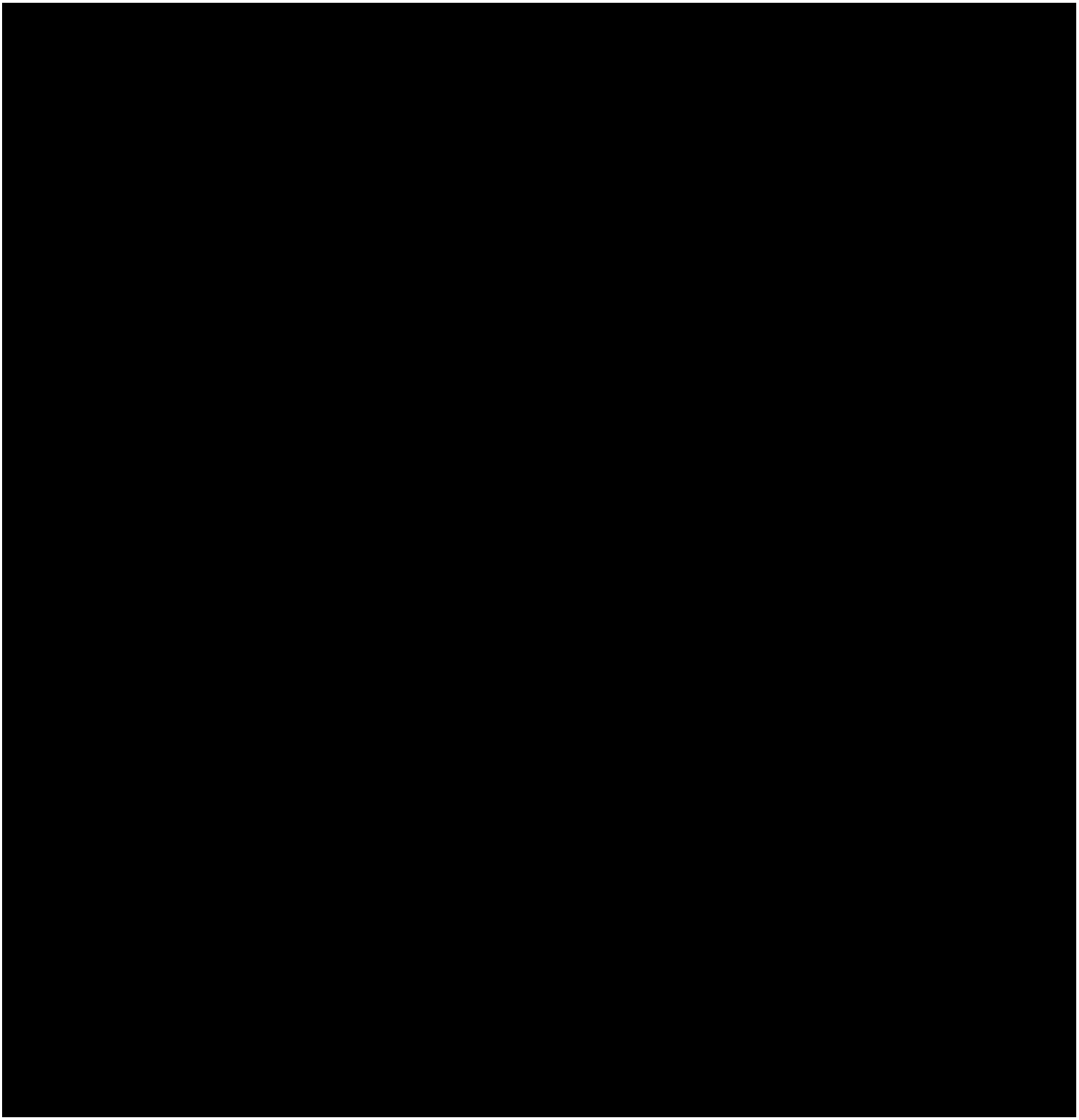


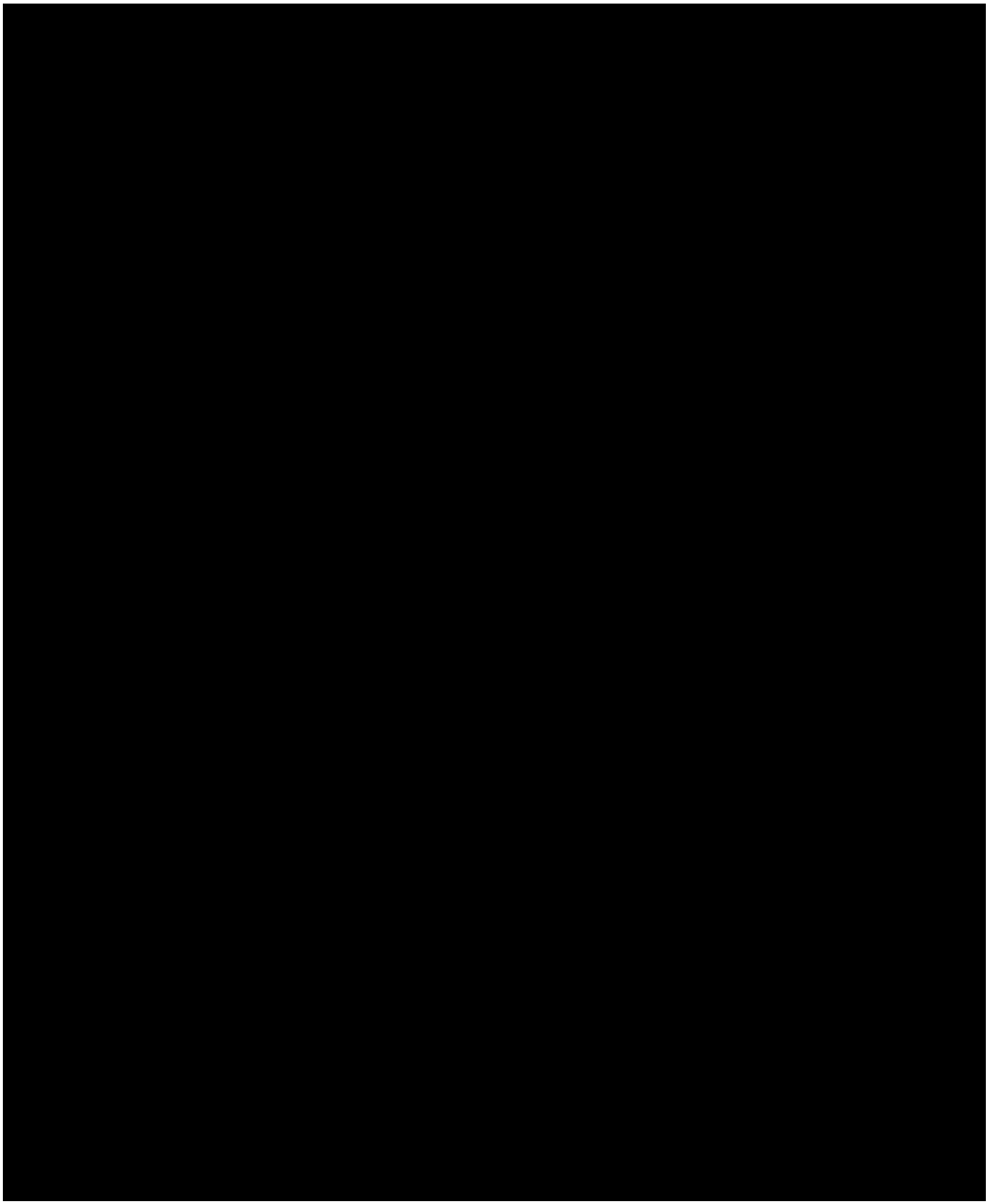












17.2 Appendix B: Schedule of Procedures/Evaluations

Evaluation	Scr	B-L	Pre-op	Infusion	BU	+1d	Months After Procedure												
							1	2	3	4	5	6	8	10	12	15	18	21	24
P.E.	X	X	-		-	X	X	X	X	X	X	X	X	X	X	X	X	X	X
History	X	X	-		-	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Comprehensive Metabolic Panel[a]	X	X	X		-	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Magnesium, phosphate	X	-	X		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CBC with diff.	X	X	X		-	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cytogenet. PB	X	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pregnancy test*	X	-	-		X	-	-	-	-	-	-	-	-	-	-	-	-	-	-
INR or PT/PTT	X	-	X		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Type and cross	-	-	X		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HIV-1, HepB & C, CMV, B19	X	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urinalysis	X	-	X		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ECG	X	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Echo	X	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CXR	X	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pulse ox.	X	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RBC dAXP	-	X	-		-	-	X	X	X	X	X	X	X	X	X	X	X	X	X
RBC ADA activity	-	X	-		-	-	X	X	X	X	X	X	X	X	X	X	X	X	X
Immune function[b]	-	X	-		-	-	X	X	X	X	X	X	X	X	X	X	X	X	X
TREC, TCRV β panel	-	X	-		-	-	-	-	-	-	-	X	-	-	X	-	X	-	X
PBMC for RCL[c]	-	X	-		-	-	-	-	X	-	-	X	-	-	X	-	-	-	X
Serum for WB to RCL[d]	-	X	-		-	-	-	-	X	-	-	X	-	-	X	-	-	-	X
PBMC for vector[c]	-	X	-		-	-	X	X	X	X	X	X[g]	X	X	X[g]	X	X[g]	X	X[g]
Busulfan PK[e]	-	-	-		X	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Myelosupp. Lab[f]	-	-	-			X	X	-	-	-	-	-	-	-	-	-	-	-	-

Scr = screen B-L = Baseline
+1d = day after infusion
BU = Busulfan: See Appendix C for details of administration
X = to be performed, - = not to be performed
*For female subject of child-bearing potential
[a]includes albumin, AST/ALT, alkaline phosphatase, total protein, total bilirubin, creatinine, BUN, glucose, calcium, Na+, K+, Cl-, total CO_2
[b]includes lymphocyte subsets phenotype, quantitative immunoglobulins, antibodies to Tet/PRP (Q 3 months if not receiving IVIg), T cell proliferation to PHA/Tet (only at 6, 12, 18, and 24 months and only to Tet if immunized against tetanus).

[c]At each time, any unused PBMC will be cryopreserved in liquid nitrogen, for possible future studies.
[d]To be banked for possible future studies
[e]Busulfan PK at 0, 1, 2, 4, 8, 13 hours following IV Busulfan administration, done during hospitalization
[f]Myelosuppression Laboratory: Hepatic function tests, CBC/Diff, 2x per week for up to 6 weeks, from day +1 until hospital discharge when neutrophil recovery is observed, or longer as indicated. For subjects who have slow recovery of counts after transplant (beyond Day +30), CBC/Diff will be performed at least once every 7-10 days until ANC is >500 cells/mm.^{3}
[g]According to current FDA guidelines, LAM-PCR samples will be obtained ≈ every 6 months, for 5 years, then annually for an additional 10 years

Schedule of Procedures/Evaluations:

I. Pre-treatment phase:

A. Screening [performed within 60 days of treatment (except for virologies, which may be performed up to 90 days)]:

The first part of the study will involve performing laboratory tests to determine whether the subject meets the inclusion criteria and does not have a contra-indication causing exclusion.

1. Complete history and physical examination including recording of height (cm), weight (kg), and vital signs (temperature, pulse rate, respiratory rate and blood pressure)
2. Blood tests:

Serum chemistries [albumin, AST/ALT, alkaline phosphatase, total protein, total bilirubin, creatinine, BUN, glucose, calcium, electrolytes (Na+, K+, Cl-, CO^2)]
Magnesium, phosphate
CBC with differential and platelet count
INR or PT/PTT
HIV-1, hepatitis B, hepatitis C, CMV, and parvovirus B19 by DNA PCR
Peripheral blood, or for neonates, cells obtained from amniocentesis, for cytogenetic analyses

3. Urinalysis: routine urine chemistry and microscopic examination
4. Electrocardiogram (EKG)
5. Echocardiogram (Echo)
6. Chest X-ray (CXR)
7. Pulse oximetry
8. Biopsy of suspicious skin lesions
9. Confirmation of ADA-deficient SCID*

*Confirmation of ADA-deficient SCID will be based upon biochemical demonstration of ADA deficiency or documented ADA gene mutation(s) known to cause disease and T and CD3+ cell lymphopenia at the time diagnosis was made.

B. Baseline measurements (performed prior to the infusion of gene-modified cells):

1. Leukocyte or erythrocyte ADA enzymatic activity and erythrocyte deoxyadenosine nucleotide levels
2. Measurement of immune function

Determination of absolute numbers of CD3, CD4 and CD8 T lymphocytes; CD19 B lymphocytes; and CD56/CD16 NK cells
Proliferative responses to PHA, tetanus toxoid
Measurement of serum immunoglobulin levels (IgG, IgA, IgM), specific antibodies to isohemagglutinins, tetanus toxoid, polyribosylphosphate {PRP}
TREC and TCRVβ panel
PBMC and serum banking for RCL determination

II. Treatment Phase: CD34+ cell isolation, transduction and transplant.

If patients meet inclusion criteria and have no findings causing exclusion, then treatment will be performed.

A. Bone marrow collection:

A.1. Pre-operative screening:

Pre-operative screening studies will be performed within three days prior to marrow harvest. These screening tests are in addition to the standard testing that each clinical site may require for their autologous hematopoietic stem cell transplant recipients. Pre-operative studies will include:

1. CBC with differential and platelets (can be used as the baseline value)
2. INR or PT/PTT
3. Magnesium, phosphate
4. Comprehensive metabolic (albumin, AST/ALT, alkaline phosphatase, total protein, creatinine, total bilirubin, calcium, BUN, glucose, electrolytes) (can be used as the baseline value
5. Type and cross for 2 units PRBC (to be done within the duration required by the blood bank at the treatment site)
6. Routine urinalysis
7. Serum HCG (pregnancy test), if female of child-bearing age

These studies must meet the inclusion criteria to allow bone marrow harvest to be performed.

A.2. Bone marrow harvest:

The patient will be brought to the operating room and be placed under general anesthesia. The region of the posterior superior iliac crests will be sterilized with Betadine™ washes and draped in a sterile field. Two physicians will simultaneously aspirate marrow from each crest, taking multiple 5-10 ml aspirates to a total volume no greater than 20 ml/kg subject body weight (maximum set by the NMDP). After collection of the marrow, sterile pressure dressings will be placed over each aspiration site, the patient will be turned over and a PICC line or central venous access device will be inserted (if a central venous access device is not already present), and the patient then awakened. Alternatively, a PICC line may be placed with appropriate sedation as a separate procedure prior to marrow harvest. The marrow will be transferred into sterile blood bags and transported to:

a) at UCLA: UCLA Bone Marrow and Stem Cell Processing Laboratory for initial processing and then brought to the GMP facility of the Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, Factor Building.

b) at the NIH: NIH Clinical Center, Building 10, Cell Processing Section (CPS) of the Department of Transfusion Medicine.

B. Bone marrow characterization:

Up to 2 ml of the bone marrow will be used to characterize the cells:

Determination of nucleated viable cell count by enumeration in a hemocytometer, coupled with trypan blue exclusion

Complete blood count with differential and platelet count

Flow cytometry analysis to measure the % CD34+ cells in the clinical lab

Bacterial culture to determine sterility

C. Bone Marrow Backup and Dosage Calculations:

C1. Bone Marrow Backup:

The volume of the harvested bone marrow corresponding to $5x10^7$/kg of total nucleated cells (TNC) or $3x10^7$/kg of mononuclear cells (MNC) from ficolled marrow will be allocated to the Bone Marrow and Stem Cell Processing Laboratory for cryopreservation according to their standard operating procedure (SOP). The TNC = the total white blood cell count (WBC) x volume of the

marrow sample; the MNC = the total cell count x volume of the ficolled sample. Characterization and cryopreservation of the back-up will be done according to the SOP of the clinical laboratory. The successful isolation of CD34+ cells from treatment phase bone marrow harvest is defined as ≥ 1 x 10^6 /kg. If between 5x10^5 and 1x10^6 cells are obtained, they will be cryopreserved as additional back-up and the subject offered a second marrow harvest. If the CD34+ cell yield from the second harvest is ≥1x10^6/kg, the cells will be transduced with the EFS-ADA lentiviral vector. If < 1 x 10^6 /kg cells are obtained from the second harvest, the subject will be withdrawn from further participation in the study.

[**NOTE:** If the subject's umbilical cord blood (UCB) was cryopreserved by a clinical stem cell lab at the time of birth, the backup cells may be allocated from the cord blood. If less than 5x10^7/kg TNC are contained in the cord blood at the time of cryopreservation, the remainder of the backup cells will be obtained from the bone marrow at the time of harvest. If 5x10^7/kg TNC are obtained from the UCB for the backup, the volume obtained from the bone marrow harvest at the time of treatment, less 2 ml required for characterization, will be used to isolate CD34+ cells for the transduction procedure.]

C2. Backup and Dosage Calculations:

A standard estimate of the minimal numbers of allogeneic bone marrow total nucleated cells used for transplant is at least 5 x 10^7 cells/kg of recipient weight. This cell number, or 3 x 10^7 MNC/kg from ficolled marrow, will be frozen as a back-up. The back-up will be used if OTL-101 does not meet infusion specifications, or if there is engraftment failure evident after +42 days post cell infusion [ANC < 200 or platelet count < 20,000, repeated and confirmed x3 total determinations). With CD34+ cells comprising approximately 1.0% of the mononuclear cells in marrow, this translates to an average number of back-up CD34+ cells being approximately 0.5 x 10^6/kg. CD34+ cells will be isolated from TNC in excess of 5 x 10^7/kg. The cells will then be transduced with the vector and, after washing, given back to the subject pending meeting the release criteria. If cells do not meet release criteria, they will not be infused. If the subject has already received Busulfan, the subject will be administered only the untransduced back-up cells.

Therefore, these dosage calculations will be used for assessment of our ability to achieve the end-points. It is difficult to specify the minimal number of transduced CD34+ cells to be infused, because it is possible that successful engraftment of transduced stem cells may be achieved with lower numbers of engrafting, transduced CD34+ cells than those set by arbitrary criteria. We consider the minimal acceptance criteria for infused, transduced cells to be 0.5 x 10^6 total cells / kg.

It should be noted that since the subjects will receive prior non-myeloablative conditioning, we will cryopreserve untransduced cells. These will be infused if the final product contains **<** 0.5 x 10^6 cells / kg. If necessary, these back-up bone marrow cells would be re-infused to overcome more severe myelosuppressive effects of the conditioning regimen (persistent ANC < 200/µl or platelets < 20,000/µl after day +42 from the day of cell infusion on three independent and consecutive determinations over at least ten days). It is recognized that there is no specific information available to extrapolate from the traditional, but empirically-based, numbers of cells needed to overcome allogeneic transplant barriers to the number of *ex vivo*-transduced, autologous cells needed for engraftment and therapeutic effects. Similarly, it should be recognized that transduction of clonogenic progenitors poorly predicts transduction of pluripotent stem cells, which

can only be assessed by transplantation into recipients and observation of cells produced over subsequent months and years.

Therefore, these observations of the numbers of CD34+ cells obtained and the percentages that become transduced will be recorded. However, if < 1 x 10^6 CD34+ cells/kg are isolated after two harvests, subjects will be withdrawn from the study and not receive busulfan. We will limit total infusion dose to 15 x 10^6 cells per kg, as approved by the FDA/CBER. If more than 15 X 10^6 cells per kg are isolated after the culturing period, the gene-modified cells that remain after the total maximum dose has been given will be disposed or stored for research.

D. Administration of Busulfan:

Subjects who meet criteria to receive Busulfan (enough cells to freeze back-up and to start culture with ≥ 1 x 10^6 CD34+ cells/kg) will remain hospitalized after the bone marrow harvest for Busulfan administration, infusion of OTL-101, and post-Busulfan monitoring (minimum of 7 days post-infusion and may be up to approximately 4 months). Subjects from out of town may be transferred after a minimum of 3 days post-infusion for post-busulfan monitoring by their home physician.

Subjects will be given a loading dose of the anti-convulsant levetiracetam (10 mg/kg IV slow infusion) prior to administration of busulfan, followed by maintenance doses of 5 mg/kg IV every day x 2 doses. Antiemetic therapy will also be started prior to busulfan therapy. Busulfan will be administered as a single IV dose (4 mg/kg) over 3 hours on day -1. Peripheral blood samples will be drawn after the busulfan infusion to measure the busulfan area-under-the-curve pharmacokinetics. At least a 24 hour "wash-out period will be observed from completion of the busulfan infusion before OTL-101 is administered on day=0. The levetiracetam will be discontinued 24 hours after the Busulfan infusion on Day= 0.

BMT day 0 marks the end of the treatment phase of the Busulfan partial marrow cytoreductive regimen. Unless otherwise indicated, anti-emetic medication will also have been discontinued. If OTL-101 meets release specifications, it is administered on this day. If OTL-101 does not meet criteria (e.g. gram or stain positive, endotoxin level too high), OTL-101 will not be given, and the back-up cells will be administered.

E. Busulfan Pharmacokinetic Determination (Refer to **Appendix C** for details):

Blood samples for Busulfan levels would be drawn in dark green-top tubes (containing sodium heparin) at the following time-points post-infusion of Busulfan: immediately following the completion of the infusion of intravenous Busulfan, and at 1, 2, 4, 8 and 13 hours after the end of the infusion. Pharmacokinetic studies will be performed in a CLIA-certified laboratory (samples may be frozen and shipped for later assay).

F. Administration of OTL-101:

A peripheral intravenous line will be established, if there is no PICC line nor a central venous catheter. Patients will be pre-medicated with an appropriate dosage of acetaminophen (10-15 mg/kg), PO and Benadryl™ (0.5-1.0 mg/kg) IV/PO, 30-60 minutes prior to infusion. OTL-101 will be infused intravenously through the central or peripheral lines into the subject. The infusion will take place in the subject's hospital room at 3F Mattel Children's Hospital at UCLA or at the 1NW Pediatric Ward of the NIH Clinical Center. The IV infusion will take less than 15 minutes.

G. Post-infusion testing (Day +1):

The subjects will remain hospitalized following the infusion and will be monitored for side effects during and after the infusion. The monitoring will include measurements of vital signs every 4 hours for 24 hours after the infusion. CBC with differential, platelet count, electrolytes, and chemistry panel will be evaluated 24 hours post-infusion.

H. Procedure for re-infusion of back-up:

Should infusion of the back-up cells become necessary, the cells will be thawed and re-infused according to the clinical laboratory standard operating procedure.

I. PEG-ADA Enzyme Replacement Discontinuation:

PEG-ADA will be stopped on day +30 post-cell product infusion if the patient received busulfan and the final cell product and does not have active infections or other major medical problems. Management of PEG-ADA ERT is more fully detailed under Section 6.4.1.

III. Post Treatment Phase

A. Sample time-points:

Follow-up studies will be performed (either as an inpatient or outpatient basis) to assess achievement of study end-points. The outpatient follow-up studies will be performed by the subject's home physician in his/her hometown for the subject's convenience. At each of the scheduled visits (see "Schedule of Investigation" above), the patients will be seen by one of the Clinical Investigators. At each visit, a complete interval history will be taken, including changes or onset of new symptoms, opportunistic infections, changes in medications, and other therapeutic modalities. Physical examinations will also be performed by a Clinical Investigator.

At months 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 21 and 24 (+/- 4 weeks), patients will have phlebotomy performed to obtain peripheral blood for studies. Studies performed at each of these time-points will include:

Safety/toxicity studies:

1. CBC with differential, platelet count
2. Serum metabolic panel, including electrolytes
3. PBMC to be analyzed at baseline, 3, 6, 12, and 24 months by PCR for RCL, then archived annually
4. Serum to be banked at baseline, 3, 6, 12, and 24 months and annually thereafter, for possible western blot testing for RCL
5. Monitoring for leukemia at baseline, and at approximately 6-month (LAM-PCR) intervals for the first five years post-infusion, then annually for an additional 10 years (15-year total monitoring after infusion) if criteria for triggering LAM-PCR are fulfilled (**see**

Appendix F for detailed Monitoring Plan for Monoclonal Expansion and Insertional Oncogenesis)

6. Measurements of erythrocyte ADA enzymatic activity and deoxyadenosine metabolites in RBC

Immune function testing:

7. Determination of absolute numbers of CD3+, CD4+ and CD8+ T lymphocytes, CD19+ B lymphocytes and CD56/CD16+ NK cells, and CD4+/CD45RA+ and CD4+/CD45RO+
8. Proliferative responses to PHA, tetanus toxoid at baseline, 6, 12, 18, and 24 months and to tetanus toxoid only after immunization to tetanus
9. Measurement of serum immunoglobulin levels (IgG, IgA, IgM)
10. Specific antibodies to tetanus toxoid, polyribosylphosphate (PRP, Q 3 months if not receiving IVIg)
11. TCR V β panel, TREC

Gene transduction/expression:

12. Measurement of the frequency of cells containing the inserted ADA gene in PBMC and granulocytes as well as FACS-sorted T cells and myeloid cells by PCR
13. Measurement of ADA expression in leukocytes and erythrocytes by ADA enzymatic assay

Blood samples to be obtained at each[1,2] post-infusion time point.

Test	Tube Type	Minimum Vol.	Destination
Serum metabolic panel, including Electrolytes	Red Top Tube	2.0 ml	Clinical Lab
CBC, differential	Purple Top Tube	1.0 ml	Clinical Lab
Erythrocyte ADA activity and dAXP	Green Top Tube (ice)	2.0 ml	Duke/Hershfield
PBMC for Lymphocyte Immunophenotype and Proliferation and TCR Vβ Usage[3]	Purple Top Tube Green Top Tube	2 X 2.0 ml 4.0 ml	Clinical Immunology Lab and Gene Therapy Core
PBMC for ADA enzyme assay (optional, for research)	Green Top Tube Purple Top Tube	8.0 ml 5 ml	Gene Therapy Core

PBMC for vector (ddPCR/qPCR and VISA), TREC			
PBMC for RCL[1]			
Serum for WB for RCL[2]	Tiger Top Tube	2.0 ml	Gene Therapy Core
Serum for Ig levels	Tiger Top Tube	2.0 ml	Clinical Lab

[1] PBMC for RCL will be done at baseline, 3, 6, 12 & 24 months, then archived annually thereafter
[2] Serum (for Western Blot [WB]) will be archived at baseline, 3, 6, 12 and 24 months, then annually thereafter
[3] Flow cytometry, NIH

Other research tests
These tests will be performed, only as appropriate, to address outcome(s) and mechanism(s) of action of the treatment. For example, we may look at T cell receptor (TCR) spectratyping from PBMCs and/or serum cytokine levels. No extra blood will be drawn for these tests; tests will be performed on existing or left-over samples from the follow-up periods defined above.
Hierarchy of tests if samples are limited:
Due to the large number of blood tests planned and the limited volumes of blood that may be obtained from young children, it may not be possible to perform every scheduled investigation at each time-point. Therefore, the hierarchy for priority of blood tests will be:

1. Safety studies: CBC w/diff, platelet count, chemistry panel & electrolytes, LAM-PCR (see Schmidt et al 2001, for description of method)
2. Monitoring ADA levels: leukocyte and erythrocyte ADA enzyme levels and RBC dAXP levels
3. Immunologic function studies
4. Gene marking/expression
5. Other research tests

B. Diagnostic bone marrow aspirates:
Bone marrow aspirates may be requested at 1 or 2 time-points in the first two years after treatment. Bone marrow samples will be used to evaluate the presence of cells containing the vector sequences by DNA-PCR of BM cells. These aspirates will be performed under conscious sedation, and parents are free to refuse these post-treatment marrow aspirates, as stated in the initial informed consent document.

C. Immunizations:
When patients have evidence of adequate immune reconstitution (absolute CD4+ $\geq$100/mm^3)at least one year after transplantation, immunization will be given with DT (or DPT, DTaP, or Tdap) and IPV every two months, for a total of three dosages. For children who have IVIg administration

stopped, vaccination will follow guidelines for post-allogeneic HSCT vaccinations as in Griffith et al, JACI 2009. If they are below normal, re-immunization with DT will be given. Annual seasonal influenza vaccination (not attenuated nasal) is recommended for subject and family.

D. Extended follow-up:

The current study plans call for follow-up for a total of two years. However, the subjects will be followed clinically beyond this point, as would any subject with an immunodeficiency disease. Standard care would also involve evaluation of immune function at least quarterly by measuring lymphocyte numbers and function (PHA, tetanus toxoid blastogenesis). Gene marking will also be evaluated as outlined in the monitoring plan, unless no gene-containing cells are seen on successive studies over twelve months. Peripheral blood serum and PBMC will be stored annually, in case assays for RCL exposure are needed. Effort will be made to continue these studies as long as possible, although it is not possible to promise life-long follow-up. The plans for life-long follow-up for the detection of RCL and the 15-year follow-up for leukemia monitoring are described in Appendix F. In the case of the death of the subjects, autopsies will be requested from appropriate next-of-kin. Tissues will be analyzed for the presence of gene-containing cells and for the presence of RCL.

17.3 Appendix C: EFS-ADA Trial Busulfan Pharmacokinetic Analysis

Purpose: Subjects will receive one dose of busulfan, @ 4 mg/kg. Busulfan blood levels will be measured following the busulfan to calculate the area-under the curve (AUC). Because only a single dose of busulfan is given, no dose adjustment will be made.

Method: Busulfan dose is Busulfex 4 mg/kg IV x 1 dose. Blood samples are taken at specified times after the dose of busulfan is infused. The busulfan levels in each sample will be measured in the laboratory and the projected net AUC calculated.

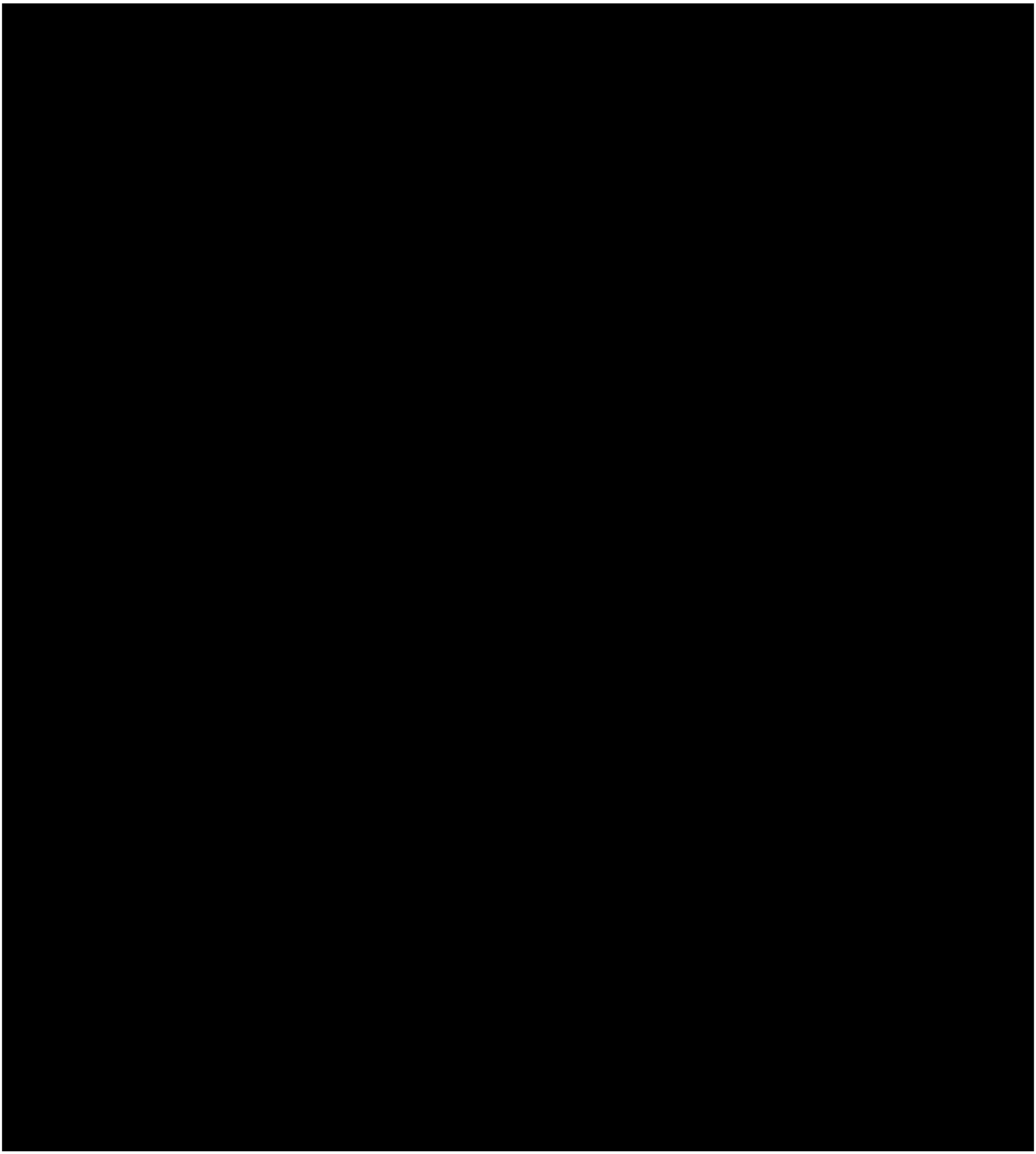
Sampling: Busulfan is administered IV over 3 hours. As soon as busulfan infusion is complete, flush CVC with 5cc NS before drawing first level. Draw first level immediately, to measure end-of-infusion peak (t=0 min). Then, draw other samples, timed from the end of the busulfan infusion: 1, 2, 4, 8, and 13 hours after the end of busulfan infusion.

Sample Times (min.)

1)	0	(immediately at end of 3 hr infusion)
2)	60	(1 hr)
3)	120	(2 hr)
4)	240	(4 hr)
5)	480	(8 hr)
6)	780	(13 hr)

The whole blood samples are processed by centrifugation and plasma fractions are frozen at -20°C until the complete set of samples for the dose are obtained.

Calculation: Plasma busulfan levels are determined by HPLC. Busulfan pharmacokinetic parameters are calculated by fitting a bi-exponential equation. Area-under-the curve (AUC) is calculated by trapezoidal approximation, while drug clearance (optional) is calculated by using the busulfan dose divided by both body weight and the measured AUC.



ANNUAL REVIEW

PRINCIPAL INVESTIGATOR	DATE

Purpose:
To ensure that hematopoietic progenitor cell products are transported in such a way as to protect the integrity of the product and the health and safety of personnel transporting the product.

Principles: Not Applicable.

Definitions:

Excipient: An inert substance used as a diluent or vehicle for a drug.

Materials:
Room temperature (20 - 25°C) gel ice pack (approx. 900 grams).
Shipping container (MiniMate Ice Chest #1 or #2) labeled “Hematopoietic Progenitor Cells” and with permanent shipping label inside and outside of container. See label example at end of procedure.
Wet ice (if applicable).
Dry ice (if applicable).
Ziplock®-type plastic bags (9” x 12”), pre-sterilized.
Liquid Nitrogen and LN_2 Shipper.

Specimen Requirements:
Whole blood, bone marrow, or Hematopoietic Progenitor Cells in excipient.

Safety Precautions:
Universal precautions are used. All patient specimens are treated as if infected by blood-borne pathogens, and therefore, gloves must be worn when handling specimens.

References:

Foundation for the Accreditation of Hematopoietic Cell Therapy (FACT) Accreditation Manual, Section D, 2nd Edition, 2002.

"Circular of Information for the Use of Cellular Therapy Products", American Association of Blood Banks, America's Blood Centers, American Red Cross, ASBMT, FACT, NMDP, ISCT, 2003.

Procedure:

Before a cell product is collected and transported, the following orders must be issued:

An order to collect the cell product.

An order to receive the cell product in the Bone Marrow (BM)/Hematopoietic Stem Cell (HPSC) Lab.

An order to release the cell product from the BM/HPSC lab to the Clinical Gene Therapy Laboratory (CGTL).

Cell Products:

Hematopoietic Progenitor Cells, Apheresis (HPC, A) is collected by the University of California, Los Angeles Pediatrics/Hematology/Oncology Department and is transported in an impenetrable container to the BM/HPSC lab (CHS 46-126) for further processing.

At the end of the apheresis collection, when prompted by COBE Spectra (or equivalent), seal and remove the Hematopoietic Progenitor Cells, Apheresis product, and plasma (if collected). Be sure the product has been labeled before disconnecting and that the label is complete.

Place Hematopoietic Progenitor Cells, Apheresis product in a secondary plastic bag and seal to prevent leakage. Place plasma, if collected, into a separate plastic bag and seal. Place any extra samples collected during and/or at the end of the procedure into a separate plastic bag and seal.

Registered Nurse (RN) or Clinical Laboratory Specialist (CLS) brings the product in shipping container, as well as accompanying paperwork, from collection site to the BM/HPSC lab to complete processing.

Hematopoietic Progenitor Cells, Marrow (HPC, M) is collected in the OR and transported in an impenetrable container (e.g. Room temperature cooler) to the BM/HPSC lab for further processing, or it is received in the BM/HPSC lab from the National Marrow Donor Program.

Hematopoietic Progenitor Cells, Umbilical Cord Blood (HPC, UCB) is delivered frozen to the BM/HPSC lab.

Transport of Hematopoietic Progenitor Cell Products, by CGTL personnel, from the procurement sites to the BM/HPSC lab (CHS 46-126) and from the BM/HPSC lab to the CGTL (Factor 14-246 or 14-650).

The product is assigned an identification number.

Place a room temperature gel ice pack and thermometer inside a MiniMate Ice Chest (or equivalent). The temperature must be between 20-25°C.
Take the transport container to the product collection site.
The cell product is released from the BM/HPSC Lab and placed in the plastic bag(s) and into the transport container.
Take the transport container with the product inside, and any relevant paperwork, to the final destination.
When the product arrives at the final destination, it is recorded in the log book, or equivalent (e.g. Transduction Worksheet).
Samples of the product are taken to determine sterility and cell enumeration (e.g. CD34+ cells).
Transport of HPC Product from CGTL to the Pediatric Heme/Onc Unit (3rd floor RRMC) for Infusion:
Infusion of HPC Product (viable, non-frozen):
After completion of specimen processing, the cells for infusion are put into a sterile 60cc syringe for infusion.
Label the syringe and place in a ziplock-type bag.
The bag containing the syringe is then placed into an empty ice chest to maintain room temperature during transport.
The syringe is taken to the infusion facility as soon as possible.
The Disposition of the Final Cellular Product (Form 1000A) will accompany the syringe to the infusion center.
The Disposition of the Final Cellular Product form is completely filled out by all individuals indicated.
When completed, the original document will be retained at the Clinical Gene Therapy Laboratory and filed in the study binder of the respective subject. Additional copies will be available upon request to regulatory agencies.
Transport of HPC Product from CGTL to Control Rate and Liquid Nitrogen Freezers, or for Dump Freezing
The HPC product is transported to the Control Rate Freezer at room temperature.
The product is frozen following SOP #6000 – Control Rate Freezer.
The product is removed from the Control Rate Freezer and placed in dry ice.
The product is taken to the Liquid Nitrogen Freezer in Factor 14-938 and stored.
The number of samples and their location is recorded in the Freezer Log Book.
For Dump Freezing, the cells are transported at room temperature to the -20°C freezer (14-246 Factor) and placed there overnight. The following day, they are placed in dry ice and moved to the Liquid Nitrogen Freezer.
The number of samples and their location is recorded in the Freezer Log Book.
Transport for Bedside Thawing:

A cart containing a Liquid Nitrogen Shipper and a 37°C water bath is prepared.
The frozen cells are transferred from the Liquid Nitrogen Freezer to the Liquid Nitrogen Shipper.
The cells are taken to the bedside of the patient and thawed in the 37°C water bath.
The cells are aspirated into a sterile syringe.
The cells are then given to the RN for infusion into the patient.

Calculations: Not Applicable.

Endpoint: Not Applicable.

Corrective Action: Not Applicable.

Attachments: Form 1000A

17.5 Appendix E: SOP for Transduction

17.6 Appendix F: Monitoring for monoclonal expansion or leukoproliferation due to insertional oncogenesis

A general protocol for investigation of possible clonal proliferations that might develop as a result of insertional oncogenesis will be followed. The purposes of the protocol are to:

Provide adequate monitoring so that determination of the need for therapeutic intervention is made as expeditiously as possible;

Minimize the risk of either physical or psychological harm of unnecessary interventions, to which subjects are exposed;

Characterize any clonal proliferation events occurring in gene therapy trials;

Determine whether any clonal proliferation resulted from insertional oncogenesis;

Characterize the clonality of the normal, genetically-modified hematopoiesis after γ-retroviral gene therapy of HSC.

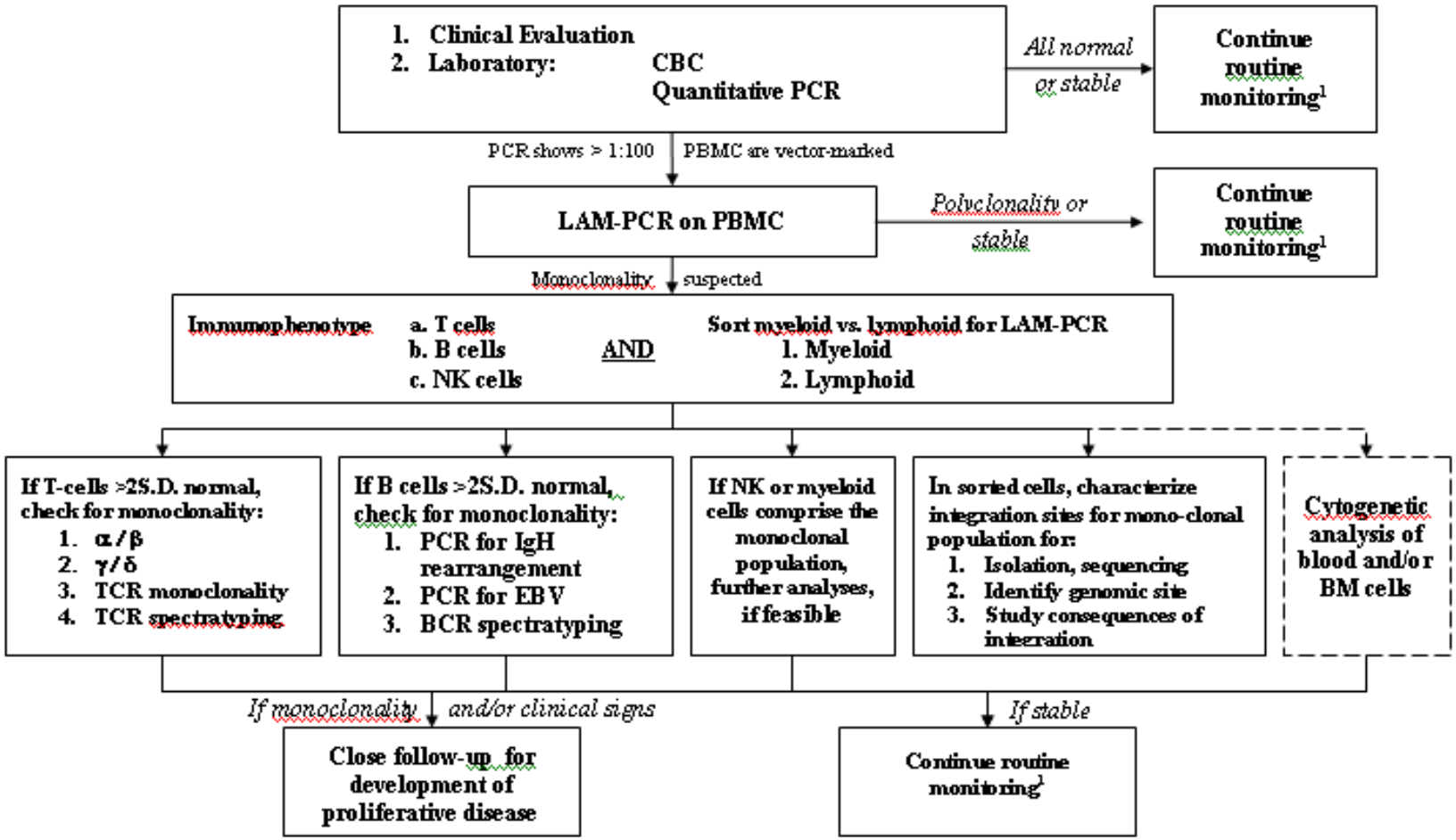
Clonal Monitoring Methods

The methods used to characterize clonal expansion in the gene therapy trials for X-SCID included LAM-PCR and TCR spectratyping. LAM-PCR (linear amplification mediated PCR) is based upon isolation of genomic DNA from peripheral blood mononuclear cells, primer extension with a 5' biotinylated primer, avidin bead isolation of the products, restriction digest to cut a 3' site, then PCR methods to amplify the antisense strand using a linker-based 3' primer. Each unique resulting band represents an individual integration site. The sequence can then be identified by searches against the human genome to determine the proviral integration site.

Information about clonality can also be gained from analyses of immunoreceptor gene rearrangements, for example, spectratyping of the TCR for T cells, and by an analogous approach for the B cell receptor (BCR) expressed by B lymphocytes. Since the TCR and Ig immunoreceptor genes are the only known genes to normally undergo rearrangement in lymphohematopoietic development, analyses of hematopoietic clonality can only be applied to the study of T and B lymphocytes, thus precluding monitoring of NK cells or non-lymphoid cells, such as myeloid cells. Furthermore, clonal lymphocyte proliferation with certain immunoreceptor rearrangements or with multiclonal rearrangements may not be detectable.

Insertional oncogenesis following gene transfer into hematopoietic stem cells could theoretically result in either lymphoid or myeloid leukemias. The techniques used to characterize the T cell lymphoproliferation occurring in X-SCID patients after gene therapy may not be adequate for other forms of malignant expansion, and it is important that monitoring protocols are identified that can be applied to all patients and possible different outcomes.

MONITORING PROPOSAL: GENE TRANSFER TO CD34+ HEMATOPOIETIC STEM CELLS



[1] Approximately every 6 months for the first 5 years on study, then annually for the next 10 years. (If at 2 years, marking is low or absent, and / or monoclonality is stable, the plan may be modified with regulatory agency guidance). CBC = complete blood count with differential; LAM-PCR = ligation/amplification mediated polymerase chain reaction; PBMC = peripheral blood mononuclear cells; S.D. = standard deviation; TCR = T cell receptor; EBV = Epstein-Barr virus; BCR = B lymphocyte receptor; BM = bone marrow.

Protocol for monitoring and evaluation of increasing oligoclonality or monoclonality:

In order to monitor subjects for evidence of uncontrolled leukocyte proliferation, the following protocol will be used:

1. Clinical Assessment

Clinical evaluation for evidence of leukocyte proliferation, or cytopenia - Physical evaluation and imaging studies (as indicated) for evidence of lymphadenopathy, hepatomegaly, splenomegaly.

2. Screening Laboratory Assessment

CBC with differential to determine whether there is evidence of leukocytosis, or cytopenia. Leukocytosis will be defined as values that are > 15,000 / mm^3 on two successive analyses, within a clinically-appropriate time-frame and in the absence of signs of infection. Cytopenia will be defined as platelets < 100,000/ul, leukocytes < 1,500/ul, or hemoglobin < 10 g/dl.

[Routine follow-up will also include serum electrolytes, creatinine, BUN, LDH, uric acid, AST, ALT, alkaline phosphatase].

Quantitative PCR for the frequency of gene-modified peripheral blood mononuclear cells (PBMC). LAM-PCR, if frequency of gene-modified PBMC > 1%.

If the LAM-PCR results suggest monoclonality, then the following laboratory assessments will be performed, as applicable:

3. Detailed Laboratory Assessment

To determine which cell population may contain an abnormally proliferating clone, immunophenotyping[1] and FACS sorting[2] will be performed on peripheral blood:

A. Immunophenotyping and determination of absolute numbers of subpopulation:

I. T lymphocytes: Cell surface markers CD3, CD4, CD8, . If the T lymphocyte number is >2 S.D. above normal values for age:

a) If there is evidence of T cell lymphocytosis (CD3), then additional immunophenotypic markers may be done to characterize the stage of differentiation, including: CD1, CD2, CD4+/45RA, CD4+/45RO, TCR α/β, TCR γ/δ,

b) If there is evidence of TCR α/β, T cell lymphocytosis, then staining for TCR Vβ usage will be performed, using a broad panel of reagents, e.g., IO Test Beta Mark ™(*Beckman Coulter*).

c) If there is evidence of TCR γ/δ T cell lymphocytosis, then TCR γ and TCR δ usage will be analyzed with a broad spectrum panel.

d) If there is an over-represented TCR family, then PCR for respective TCR monoclonality will be performed.

e) TCR spectratyping may also be used to assess/confirm oligo-monoclonality.

II. B lymphocytes: Cell surface markers CD19 and CD20, Igκ and Igλ If there is evidence of abnormal distribution of Igκ or Igλ expression, then IgM, IgD, IgG, IgA staining may also be performed to determine if a clonal B cell population is present.
If the B lymphocyte number is >2 S.D. above normal values for age:

a) If there is evidence of B cell lymphocytosis (CD19 or CD20), then additional immunophenotypic markers may be done to characterize the stage of differentiation, inclujding: CD10, CD27,

[1] These assays are expected to require 2-5 ml of blood, depending on the WBC count
[2] These assays are expected to require 5-30 ml of blood, depending on the WBC count

b) Perform PCR or Southern blot for IgH rearrangement to characterize multiple myeloma, macroglobulinemia and lymphoma, for example.
c) Perform PCR for EBV (to evaluate for Epstein-Barr infection).
d) BCR spectratyping may also be used to assess/confirm oligo-monoclonality.

III. NK cells: Cell surface markers CD16, CD56, CD57, CD94, CD158, CD3-negative. If NK or myeloid cells are sustaining the clonal expansion, however, further immunophenotypic assays of the clonality of the hematopoietic lineage are likely to be difficult or impossible, as previous studies have depended on X-inactivation in females or the presence of specific mutations or cytogenetic abnormalities. LAM-PCR integration site analysis of total peripheral blood leukocytes and sorted myeloid cells will be performed to track clonal changes sustained by these cell types. However, marrow analysis may be useful in determining whether hematopoietic differentiation is normal or not. Marrow examination may also be required if there is significant leukocytosis or cytopenia.

B. Sorting (if sufficient cells) for LAM-PCR:

I. Lymphoid cells: Surface markers CD3 for T-cells and, CD19 for B-lymphocytes.

II. Myeloid cells: Surface markers CD33, CD13, and/or CD14.

Genomic material extracted from sorted cells will be used to characterize the site of integration of the gene transfer vector:
Isolation and sequencing of the predominant LAM-PCR fragment.
Identification of the genomic location of integration.
Attempt to study the consequences of the integration event in terms of induction or suppression of gene expression.

III. High resolution cytogenetic analyses of blood and/or marrow cells, as indicated. These analyses can be performed in the presence of any type of leukocyte clonal proliferation and would complement the above studies.
Monitoring for monoclonal expansion or leukoproliferation due to insertional oncogenesis

17.7 Appendix G: Toxicity Table For Grading Severity Of Adverse Experiences (>3 Months Of Age)

17.8 Appendix H: Details of historical HSCT control groups

1. Objective

Historical data from ADA-SCID patients who were treated with Hematopoietic Stem Cell Transplantation (HSCT) at GOSH/UCL (UK) or Duke University Children's Hospital (USA) during the period of 2000-2016 will be collected. HSCT currently corresponds to the standard of care for ADA-SCID patients. These data will be used as historical control groups in a current on-going gene therapy study "*Phase I/II, controlled, open-label, non-randomised, single-centre trial to assess the safety and efficacy of EF1αS-ADA lentiviral vector mediated gene modification of autologous CD34+ cells from ADA-deficient individuals*". The objective is to compare gene therapy versus HSCT to determine the optimal treatment.

2. Study populations

The control groups will be constituted of patients of 0 to 18 years of age with a confirmed diagnosis of ADA deficiency, transplanted at Great Ormond Street Hospital (UK) or Duke University Children's Hospital (USA) between 2000 and 2016. See inclusion criteria page 64.

3. Data to be collected – GOSH/UCL

GOSH hold a registry / clinical database (European Society for Immunodeficiencies – ESID and European Bone Marrow Transplant Registry - EBMT) displaying information on patients who have received HSCTs. 25 treated patients have been identified between 2000-2016. The patients have given consent for their data to be entered into this registry / clinical database and to be used for other ethically approved studies. ESID and EBMT will allow us to collect the below information:

- Demographics (gender, date of birth, date of death),
- Date of treatment with HSCT
- Method of ADA-SCID diagnosis,
- Previous HSCT including: date, transplant cells, donor type and outcome and whether they needed a second transplant.

However, to make this historical control group valid for marketing authorisation application, our discussions with regulatory agencies suggests that we need to collect further information valuable to compare the efficacy and safety of any new treatment in research vs. current HSCT standard of care as:

- Previous PEG-ADA ERT (and duration of treatment),
- Previous or current immunoglobulin therapy,
- Listing of relevant medical history, including infections (severe or opportunistic infections)
- Vaccination
- Auto-immune disease
- Oncology disease / leukemia
- AEs and SAEs

- Death and reason for death

This information is only available from the patient's records; we therefore plan to re-consent these patients to access their full medical records, and share the information with regulatory agencies and pharmaceutical companies.

4. Data to be collected – Duke University Children's Hospital

Duke University maintain a database of information on patients who have received HSCTs. 10 treated patients have been identified between 2000-2016. The patients have previously given consent to their data being entered into this database and being used for other ethically approved studies.

The following data will be collected from this control group:

- Demographics (gender, year of birth, age at presentation to site),
- Diagnosis (age at and method of ADA-SCID diagnosis),
- HSCT history, including: age at transplant, type of transplant cells and number, donor type and outcome, need for a second transplant, outcome of vaccinations post-transplant,
- Use of PEG-ADA ERT and immunoglobulin therapy (before and after transplant), including time since transplant if reinitiated post HSCT,
- Graft versus host disease,
- Listing of relevant medical history, including infections (severe, recurrent or opportunistic infections) and neurological conditions,
- Previous and Concomitant Treatment,
- Changes in Medical Condition post HSCT, including severe/opportunistic infections, emergent neurological events, autoimmune conditions, oncologic disease, SAEs, bone/skeletal deformities, physical developmental delays or weight loss,
- Lymphocyte subsets (CD3, CD4, CD8, CD19, CD56/CD16 and CD4+/CD45RA cell counts) at baseline and at 1-8 months, 9-16 months and 17-30 months post HSCT,
- ADA activity & ADA metabolites (plasma deoxyadenosine, ADA activity in RBCs, dAXP) at baseline and at 1-8 months, 9-16 months and 17-30 months post HSCT,
- Death and reason for death.

5. Data collection process

This is a retrospective, non-interventional data collection activity; the patients follow routine medical practice. We will contact GOSH patients or their authorised representative and seek their consent to get access to their medical notes and share the information with regulatory agencies and pharmaceutical companies. Teams at GOSH/UCL and Duke University Children's Hospital will work on the collection of data from databases and patients' records, and the data will be entered into an electronic Case Report Form (eCRF). This eCRF will be monitored as per standard process before it can be used for statistical analysis and submission to agencies for review and assessment.

6. Data Analysis

The comparison analysis between gene therapy and HSCT will be done based on the current gene therapy study objective:
Survival and event free survival, defined as need to return to PEG-ADA or second transplant at 1 and 2 years post treatment.
Use of immunoglobulin replacement therapy (IgRT) and severe infections post treatment.
Severe infections, defined as infections requiring hospitalisation or prolonging hospitalization and/or documented infections by opportunistic pathogens (interstitial pneumonia, intractable diarrhoea)

17.9 Appendix I: List of Protocol Changes

Changes made 23 April 2013 (ver. 1.3) from previous version dated 8 October 2012 (ver. 1.2)

1. Remove measurement of plasma ADA levels as follow-up test
   Reason: subjects are withdrawn from PEG-ADA ERT 30 days post infusion of gene-modified cells, and thus, this test would not be informative
   Protocol pages affected: 51, 66, 71, 72, 110, 111, 117

2. Remove ophthalmologic exam from baseline screen
   Reason: not needed
   Protocol pages affected: 70, 110, 111

3. Remove CBC test from Day 0 (Day of Infusion)
   Reason: not needed, subjects already have a CBC drawn on Day +1
   Protocol pages affected: 72

4. Subjects who discontinue study will be replaced (previously were not replaced)
   Reason: would allow treatment of 10 subjects
   Protocol pages affected: 80

5. Add identity test to characterization of final product
   Reason: per FDA request
   Protocol pages affected: 57

6. Change criteria for reinstituting PEG-ADA ERT
   Reason: clarification, to prevent prematurely re-starting PEG-ADA based on previous study data
   Protocol pages affected: 62

7. Add TCR Vβ panel assay to schedule of procedures
   Reason: correction
   Protocol pages affected: 110

8. Remove vector marking test from Day +1 (Schedule of Procedures)
   Reason: not needed
   Protocol pages affected: 110

9. Change description of allocating the back-up bone marrow
   Reason: clarification based on technical procedures at UCLA
   Protocol pages affected: 14, 56, 58, 64, 113, 114

10. Correct time-points for blood collection of samples for busulfan pharmacokinetics
    Reason: consistency
    Protocol pages affected: 71, 115

11. Add a scientific reference
    Reason: update
    Protocol pages affected: 30, 103

12. Update Appendices C, E, and G
    Reason: update
    Protocol pages affected: 120, 126, 131

Changes made 13 May 2013 (ver. 1.4) from previous version dated 23 April 2013

1. Clarified the objectives
   Reason: to emphasize that safety is the primary objective
   Protocol pages affected: 11, 42, 47, 54, 62, 71, 80

2. Added IND #15440 to sample label
   Reason: update
   Protocol page affected: 59

3. Correct grammar (quantitation)
   Reason: correction
   Protocol page affected: 81

Changes made 03 July 2013 (ver. 1.4a) from previous version dated 13 May 2013

1. Added Hepatitis C as screening virology test
   Reason: to be consistent with informed consent
   Protocol pages affected: 52, 70, 111, 112
2. Change anti-epileptic drug prior to busulfan administration from dilantin/fenytoin to Keppra
   Reason: hospital procedure
   Protocol pages affected: 62, 72, 116

Changes made 01 October 2013 (ver. 2) from previous version dated 03 July 2013

1. Change Inclusion/Exclusion criteria to allow for different reference ranges established by different diagnostic labs and to align SCID definition with PIDTC guidelines
   Reason: update
   Protocol pages affected: 53, 114
2. Change the duration of busulfan administration from 2 to 3 hours
   Reason: to be consistent with hospital practice
   Protocol pages affected: 72, 118, Appendix C
3. Change the trade name drug “Keppra” to its generic name, levetiracetam
   Reason: to allow clinical sites flexibility
   Protocol pages affected: 73, 117
4. Specify the tests in chemistry and immune function panels and describe other tests
   Reason: clarification
   Protocol pages affected: 71, 112-114, 115, 120
5. Exclude DFSP as a malignancy that would halt enrollment because some ADA-deficient SCID patients will develop DFSP, and DFSP is not an exclusion criterion
   Reason: clarification
   Protocol page affected: 81, 87
6. Change the window for follow-up visits to be +/- 4 weeks to be consistent throughout the protocol
   Reason: consistency
   Protocol page affected: 119

Changes made 10 February 2014 (ver. 3) from previous version dated 01 October 2013

1. Allow for subjects to be transferred to the care of their home physician for monitoring 3 days after infusion of gene-modified cells
   Reason: to allow for flexibility for out-of-town subjects and their families
   Protocol pages affected: 65, 75, 119
2. Added additional investigators involved in patient care
   Reason: add to protocol
   Protocol pages affected: 17, 111
3. Correct error in time of busulfan administration (from 2h to 3h)
   Reason: error
   Protocol page affected: 123

Changes made 4 June 2014 (ver. 3.1) from previous version dated 10 February 2014

1. Change: Update "Table of Contents"
   Reason: For accurate pagination
   Protocol pages affected: iv-vii

2. Change: Reformat section titles and/or tables and/or margins
   Reason: Change format for clarity, logical pagination and "Table of Contents" update function to work properly
   Protocol pages affected: 10-12, 15-16, 18-19, 23, 107-108, 111-112, 114-115, 119-120, 123-125, 130-131, 135-136

3. Change: Updated investigators involved in patient care and updated organizational chart
   Reason: Change in personnel and added new investigators to protocol
   Protocol pages affected: 16, 17, 109, 113

4. Change: Add PBMC ADA Activity to the Schedule of Evaluations on page 114
   Reason: Correction; test was omitted
   Protocol pages affected: 113-114

Changes made 13 January 2015 (ver. 4.0) from previous version dated 4 June 2014

1. Increase number of subjects from 10 to 15
   Reason: Enrollment of 10 subjects was fulfilled and more subjects have been identified. Preliminary data indicates that the protocol is safe and efficacious.
   Protocol pages affected: 11, 45, 53, 88

3. Add prior allogeneic HSCT as an exclusion criteria
   Reason: do not want to enroll subjects who have had previous exposure to chemotherapy
   Protocol pages affected: 55

4. Update prophylactic medications requirements
   Reason: to allow for reasonable clinical management of subjects
   Protocol pages affected: 64-65, 67-68

5. Revise statistics due to increasing the number of subjects to 15
   Reason: update
   Protocol pages affected: 88

6. Add additional clinicians involved in subject follow-up
   Reason: update
   Protocol pages affected: 114

7. Clarify when T cell proliferative studies to PHA and tetanus should be conducted
   Reason: clarification, test not necessary at every follow-up time point
   Protocol pages affected: 115-116, 123

8. Clarify immunization schedule of treated subjects
   Reason: to allow for reasonable clinical management of subjects
   Protocol page affected: 124

9. Correct the volume of bone marrow that may be harvested. There was inconsistency in 2 places.
   Reason: correct inconsistency
   Protocol page affected: 74

Changes made 02 June 2015 (version 5.0) from previous version 4.0, 13 January 2015

1. Add that international normalized ratio (INR) may be used instead of prothrombin time (PT) for screening; INR is standard for coagulation studies
   Reason: update
   Protocol pages affected: 55, 72, 73, 116, 118

2. Increase screening window to 60 days and make consistent throughout protocol
   Reason: window was too narrow and caused protocol deviations
   Protocol pages affected: 54-55, 72

3. Increase the number of subjects to 20 and correct statistics
   Reason: enrollment into the trial has occurred faster than anticipated. An additional 2 subjects (past 15) have been preliminarily identified

Protocol pages affected: 11, 45, 53, 85, 88

4. Correct the flask size for cell processing
   Reason: error
   Protocol page affected: 59

5. Change when TMP/SMX may be resumed after busulfan
   Reason: clinically OK to start when neutrophils have recovered to 500
   Protocol page affected: 68

6. Update clinicians involved in subject follow-up/care
   Reason: update
   Protocol pages affected: 114-115

Changes made 06 October 2015 (version 6) from previous version 5.0 02 June 2015

1. Update organizational chart
   Reason: update
   Protocol page affected: 18

2. Change the window for follow-up visits to be +/- 4 weeks to be consistent throughout the protocol
   Reason: consistency
   Protocol page affected: 51

3. Change method of ADA enzyme measurement in PBMC from the NIH, CLIA-quality TLC method to UCLA, research-quality Diazyme colorimetric method. Also add that erythrocyte ADA enzyme levels (from CLIA approved lab, Duke University) will also be recorded.
   [redacted]
   Protocol pages affected: 52-53, 64, 69-70, 87-88, 90-91.

4. Add that lymphocyte proliferation assay may be performed using ^{3}H thymidine or CFSE
   Reason: most labs are moving away from the radioactive assay to dye-based methods that can be measured on a flow cytometer
   Protocol pages affected: 53, 70.

5. Add that confirmation of ADA enzyme deficiency can also include documentation of gene mutation known to cause disease
   Reason: clarification
   Protocol pages affected: 54, 73, Appendix B.

6. Clarify the infection status eligibility for enrollment
   From: "Evidence of active opportunistic infection or infection with HIV-1, hepatitis B, Hepatitis C, CMV, or parvovirus B 19 by DNA PCR within 60-90 days prior to bone marrow harvest."
   To: "Evidence of infection with HIV-1, hepatitis B, Hepatitis C, or parvovirus B 19 by DNA PCR within 90 days prior to bone marrow harvest. If other infection is present, it must be under control (e.g. stable or decreasing viral load) at the time of screening."
   Reason: clarification
   Protocol pages affected: 55

7. Remove fungal stain requirement for final product release
   Reason: fungal stain is not a requirement by the FDA for product release. It was added at the request of the UCLA IRB. However, including the stain delays the release of the final product (which is infused fresh) because the central lab at RRMC does not perform the test. The sample has to be driven to the Brentwood Annex for the stain. Additionally, the gram stain will also stain fungi, if present.
   Protocol pages affected: 59-60, Appendix B.

8. Add that for vector copy number (VCN) determination, digital droplet PCR (ddPCR) may be used
   Reason: update of procedures
   Protocol pages affected: 60, 65, 69-70, 83

9. Change the method of mycoplasma detection from the culture method to PCR
   Reason: update to more rapid PCR method at Labs, Inc, which is a CLIA-accredited, FDA-registered, and TGA GMP-compliant laboratory
   Protocol page affected: 60

10. Update PEG-ADA re-starting criteria to include change in measurement of ADA enzyme activity
    Reason: to coordinate protocol procedures to be consistent with new changes
    Protocol page affected: 64

11. Correct the tests included in a chemistry panel to be consistent throughout the protocol
    Reason: correction/consistency
    Protocol pages affected: 69, 73, Appendix B

12. Correct γ-retro to lentivirus
    Reason: correct a mistake
    Protocol page affected: 71

13. Remove requirement for recording height and weight at Day +1 after infusion, since it is already recorded at screening and baseline
    Reason: not needed
    Protocol page affected: 73, Appendix B

14. Correct blood draw times for determination of busulfan levels to be consistent with previous change
    Reason: correct a mistake
    Protocol page affected: 75

15. Coordinate descriptions of follow-up exams to be consistent with Schedule of Procedures (Appendix B)
    Reason: clarification
    Protocol pages affected: 75-76, Appendix B

16. Update DSMB reporting requirements
    Reason: update based on DSMB schedule
    Protocol page affected: 84

17. Update data handling chart
    Reason: update
    Protocol page affected: 94

18. Update monitoring plan and responsibilities, delete duplicate paragraphs and sections noted
    Reason: Update and correction. The trial enrolls a small number of subjects (up to 20) and thus monthly statistical analysis is excessive.
    Protocol pages affected: 94-98.

19. Update list of clinicians involved in patient care

Reason: update
Protocol page affected: 17, 116

20. Update scientific references
Reason: update
Protocol pages affected: 26, 106.

Changes made 02 November 2015 (version 7) from previous version 6, 06 October 2015

1. Allow ANC ≥500 cells/mm^3 alone to be acceptable inclusion criterion without the need for analysis of bone marrow biopsy/aspirate.
Reason: not necessary based on previous cases
Protocol page affected: 55

2. Clarify schedule for collecting myelosuppression labs; does not need to be for 6 weeks, if not clinically indicated.
Reason: consistency, clarification
Protocol pages affected: 51, Appendix B

3. Add monitor
Reason: update
Protocol page affected: 109

4. Update physicians involved in patient follow-up
Reason: update
Protocol page affected: 112

Changes made 30 March, 2016 (version 8) from previous version 02 November, 2015

1. Specify on the cell product label that the number of cells is total nucleated cells
Reason: clarification
Protocol page affected: 62

2. Add additional lab monitoring (weekly CBC/diff) of subjects who are slow to recover their cell counts until ANC is >500 cells/mm^3
Reason: DSMB request
Protocol pages affected: 68, 117

3. Modify study stopping criteria

   FROM:
   If there is one death or two grade 4 toxicities (except grade IV busulfan-related hematologic serious adverse events {transient leukopenia, anemia, thrombocytopenia resolving within 42 days of transplant}, which are anticipated and will be reported, but will not be considered stopping criteria).

   TO:
   If there is one death or four grade 4 toxicities, (except grade IV busulfan-related hematologic serious adverse events {transient leukopenia, anemia, thrombocytopenia resolving within 42 days of transplant} which are anticipated and will be reported, but will not be considered stopping criteria). Additionally, neutropenia (a documented disorder of ADA-deficiency) occurring at any time, will be graded for severity but will not count towards the stopping rule.
   Reason: change
   Protocol pages affected: 83, 89-90

[redacted]

Changes made 21 October 2016 (version 9) from previous version 8, 30 March 2016
(pages referenced correspond to “tracked” protocol version)

1. Name investigational product “OTL-101” for easy reference
   Reason: clarification
   Pages affected: throughout document

2. Remove PBMC ADA activity from efficacy analysis
   Reason: the PBMC ADA activity assay is research quality. We will use erythrocyte ADA activity, which is performed in CLIA lab at Duke, instead
   Pages affected: 16, 124, 131, Appendix B

3. Update results of the γ-retroviral trial (MND-ADA)
   Reason: update
   Pages affected: 36-37

4. Update results and scientific references of insertional oncogenic events in other trials using retrovirus
   Reason: update
   Page affected: 39

5. Update other clinical trials using lentiviral vectors
   Reason: update
   Page affected: 41

6. Revised Trial Description and Study Objectives
   Reason: revised to match updated statistical section
   Pages affected: 52-57, 71

7. Update method of mycoplasma testing used for release of cell product
   Reason: update
   Page affected 63

8. Update DSMB
   Reason: update
   Page affected: 87

9. Update/revise statistics section (Section 11)
   Reason: revise and update
   Pages affected: 87-100

10. Update data monitoring procedures
    Reason: change from 80% to 100% source document verification
    Page affected: 102

11. Update list of clinicians involved in patient care
    Reason: update
    Page affected: 123

12. Added Appendix for description of HSCT control arm. Reordered appendices to accept new addition
    Reason: addition to align with new objectives/statistics sections
    Pages affected: appendices H and I

Changes made 18 April 2017 (version 10) from previous version 9, 21 October 2016
(pages referenced correspond to “tracked” protocol version)

[redacted]

2. Addition of level 4 sub-headers to sections 2 and 4.
   Reason: administrative reasons allowing the headings to be part of table of contents.
   Pages: Sections 2 and 4.

3. Primary objectives reworded and presented as separate bullets for ease of reading.
   Reason: administrative change
   Page: 12 (protocol summary) and 53

4. Secondary objectives, sub-headings of primary and secondary efficacy ave been removed and a list of secondary objectives are presented and aligned with the statistics section (Section 11). These revisions have also been aligned with the endpoints.
   Reason: to align the text of objectives and endpoints with the statistical section
   Pages: 12 (protocol summary), 53, 55 and 56.

5. Effects on ADA reconstitution on immune phenotype and function endpoint, text from introduction is bulleted as endpoint 11.
   Secondary Efficacy End-point 11: Infection rates will be also measured under immune reconstitution as part of the efficacy assessment of a competent immune system.
   Reason: administrative change.
   Page: 57

6. Text added: Signed written informed consent according to guidelines of the IRB (UCLA Office of Human Research Protection Program and National Human Genome Research Institute (NHGRI) Institutional Review Board.
   Reason: administrative change.
   Page: 59.

7. Added correct level 2 sub-headings to Section 7 in order for these headers to be capured in the table of contents.
Reason: administrative change.
Pages: 70 to 73.

8. Abbreviation correction for Electrocardiogram (EKG changed to ECG).
Reason: administrative change.
Page: 77.

9. Updated information for reporting safety events to the DSMB in section 9.5.2.3 and 14.5, due to study oversight being transferred to the UCLA CTSI DSMB in September 2016.
Reason: to comply with UCLA CTSI DSMB standard procedures.
Page: 86 and 102.

10. The statistical considerations section of the protocol has been updated to be consistent with the statistical analysis plan generated for the study. This includes defining what data will be reviewed at the interim analysis at 6 months and the final analysis. The text is also refined to match the study objectives and endpoints, and distinguishes the different populations to be used for the safety and efficacy analyses.
Reason: Changes to Section 11 (Statistical considerations) to be inline with the statistical analysis plan, the objectives, and endpoints of the study.
Pages: 89 to 95.

11. The auditor has changed from UCLA Office of Research Compliance to Clinical and Translational Science Institute, and all audit findings will be submitted to UCLA IRB, rather than both NIH and UCLA IRBs.
Reason: Administrative change.
Page: 99.

12. Added correct level 2 sub-headings to Section 17 (Appendices) in order for these headers to be capured in the table of contents.
Reason: administrative change.
Pages: 114 to 146.

Changes made 13 November 2017 (version 11) from previous version 10, 18 April 2017
(pages referenced correspond to “tracked” protocol version)

1. Point viii was updated to note that obligations for the conduct of the study have been transferred by the IND holder to the CRO, TCTC.
   Reason: Updated following involvement of the CRO, to be in line with standard practice.
   Page: ii

2. The header for Section 12 of the protocol was added to the Table of contents.
   Reason: Administrative change.
   Page: vi, 91.

3. A provision was added for collecting limited long-term data for withdrawn patients
   Reason: tracking the survival of withdrawn patients, as well as their need for a rescue HSCT or reinstitution of PEG-ADA ERT, allows to collect better quality trial data.
   Page: 75, 82, 83.

4. It was clarified that participants will be asked to continue scheduled evaluations if voluntary withdrawal occurs, post-treatment only.
   Reason: Updated for clarity.
   Page: 75 and 83.

5. The information to be collected in the CRF for all AEs was clarified, and a seriousness assessment was added to the list of fields to be completed for each AE. It was also noted that grade 3 and 4 AEs should be reported to the Sponsor, as well as to the PI or Study Coordinator, within 48 hours. Leukoproliferation was noted to be an “SAE of special interest” rather than an “expected SAE”, as it has never been observed in relation to OTL-101 so far.
   Reason: Updated for clarity.
   Page: 78 and 79.

6. Causality of Serious Adverse events will now be defined as definitely related, probably related, possibly related, unlikely related, or not related to the study agent. The text was also updated to more precisely define the nature of certain events, as Serious Adverse Reactions rather than SAEs.
   Reason: Updated to be in line with standard practice.
   Page: 80.

7. The language on the RAC reporting requirements was updated to be in line with current regulations. The word “expected” was removed as considered to refer only to adverse reactions or serious adverse reactions, and modified to “anticipated”.
Reason: Updated to be in line with currnt regulations and standard practice.
Page: 81.

8. The Statistical Considerations section (Section 11) was updated to include the Duke University HSCT control group, as well as to align the language in the section to the updated Statistical Analysis Plan. Use of the Duke University HSCT group as a comparator for the trial was agreed with the FDA at the End of Phase II meeting held on 29Sep2017.
Reason: The Duke HSCT control group was added to reflect FDA feedback, and the language modified to match the updated Statistical Analysis Plan.
Page: 84 to 88.

9. The text was updated to eliminate the requirement for completed eCRF pages to be printed and stored in the patients’ medical records, as this was burdensome for the site and the monitors.
Reason: Updated to reduce the burden on site and monitors.
Page: 93.

10. The name and email address of the Regulatory Coordinator were updated.
Reason: Administrative change.
Page:109.

11. Name and addresses were added for vendors involved in the study (GeneWERK, TechnoSTAT and Diamond Pharmacovigilance).
Reason: Administrative change.
Page: 112 and 113.

12. Appendix H was updated to include the Duke University HSCT control group, and to describe what data will be collected for this patient population. Use of this patient group as a comparator for the trial was agreed with the FDA at the End of Phase II meeting held on 29Sep2017. The paragraph regarding GOSH HSCT patients was updated to reflect the fact that 25 subjects treated between 2000 and 2016 were identified at GOSH, rather than 29. The Data Analysis section was also updated to clarify that severe infections will be considered in the comparison between trial data and HSCT data, rather than all infections.

Reason: the Duke HSCT control group was added to reflect FDA feedback; the number of GOSH HSCT patients identified was corrected; the data analysis section was updated for clarity.
Page: 139 to 141.

Changes made 28 March 2018 (version 12) from previous version 11, 13 November 2017
(pages referenced correspond to “tracked” protocol version)